

nature

June 19, 1975

**Tom is now a great man of science . . .
and knows everything about everything,
except why a hen's egg don't turn into a crocodile.**

The Water Babies, Charles Kingsley

THE BBC Science Consultative Group reports to the Annan Committee on the Future of Broadcasting that a 'Dial-a-Scientist' radio programme for adults might be introduced.

ANNOUNCER: In the studio with me this evening to answer listeners' questions we have, first, Professor Roughly Nozall. In addition to his chair in polymathy at Princeton he is visiting professor in ecosystems at Hawaii, in cybernetics at Moscow, and in ethics at Paris. He is at present on a Pan-Am Travelling Fellowship. Next is Dr Vude-Narrowly, reader in theoretical achromatic optical surfaces at the University of Stoke-on-Trent. Finally our resident pundit, Dr Trout.

The lines are open, so let's hear from you. Mr A?

MR A: Does cigarette smoking cause cancer?

ANNOUNCER: Nozall?

NOZALL: That's a terribly good question. I think I can reasonably assert that scientists are devoting an ever-increasing amount of time to studying this matter and will not fail to come up with an answer. Of course it largely depends on the meaning of the words. I mean, 'cigarette smoking'—does that include cigars, does it include those who don't inhale? And 'cancer'—do we have a clear concept of the exact definition of cancer? And (*warming to his task*) what, for that matter, does 'cause' mean? I mean, some say that cancer may dispose people to smoke if you see what I mean, Mr A.

MR A: Er, yes.

VUDE-NARROWLY: I would counsel extreme caution. Until there are better microscopic techniques with clearer achromatic images at ultra-high magnification—I must add in parenthesis that the government has been exceedingly dilatory in supporting such work—it is almost impossible to get to grips with the small-scale particles undoubtedly tied up with the causative processes. It would be awfully wrong of us to give you a cut-and-dried answer in the meantime.

TROUT: Well you chaps can doubtless give technical answers, but what Mr A and probably millions of others really want to know is 'should I try to give it up?' Of course, if you can give it up do, because it's expensive; you hardly need me to tell you that. If you can't, well, just try and keep your habit to yourself—don't smoke in non-smoking compartments, don't drop fag-ends down the toilet and don't blame me if you can't stop coughing—why, only the other day . . .

ANNOUNCER: I think we'd better move on. Mrs B?

MRS B: Do black holes exist?

VUDE-NARROWLY: A terribly good question. You see the problem we scientists face is in seeing something that is black. However fine the telescopes, and I must say that although our telescopes are fine, there is a lot of room for

improvement and an inadequate resolve amongst the purse-string-holders to finance such improvement, well as I was saying, seeing black things, even inferring their existence is just very difficult.

TROUT: If I might break in, I suspect Mrs B might be worried about these weird things and what threat they might pose to us with their event horizons and all that sort of stuff . . .

MRS B: Well actually I . . .

TROUT: Just so. Don't worry, Mrs B. In our lifetimes you can forget about them. They're light years away and . . .

NOZALL (*an encyclopedia now open in front of him*): Well that's puzzling, Trout, what about the one in Calcutta, which if I remember rightly was . . .

ANNOUNCER: Perhaps we should move to Mr C.

MR C (*very faint*): My mates at work **click click click click** science has not helped humans **click click click** I reckon there is such a thing as progress **click click click** scientists think?

ANNOUNCER: I'm sorry, Mr C, there always seem to be bad connections these days, I'll have to switch to Mr D.

MR D: If a mirror changes left to right, why doesn't it change top to bottom?

(*The silence is broken only by the sound of Nozall desperately hunting in reference books. Finally . . .*)

ANNOUNCER: Well, Trout, you're not often lost for words.

TROUT: It's a terribly good question and one that all sorts of people ask. I think I'll leave it for an optics expert. Vude-Narrowly?

VUDE-NARROWLY: Well it really is out of my field—you see I deal with the transmission of light, not its reflection, which may sound a fine distinction but . . .

NOZALL (*in great excitement*): Yes, it says here, er I mean, I've always thought it was because for an outside observer to compare a man with his reflection necessitates a half rotation of the man to face the observer. This is conventionally and most easily done about a vertical axis and thus we talk of transposing left and right; although it could also be done by rotation about a horizontal axis in which case we would be forced to admit that top had become bottom but not left right, so to say.

ANNOUNCER: Mr D seems to have rung off. Time for one more! Mrs E.

MRS E: I'm not a scientist but in the last 25 years I've been putting my thoughts on paper, and now I have a manuscript of 200 pages on my theory of the origin of the Universe and another 300 pages on the nature of time, and another of 400 pages on my theory of cancer—all illustrated with colour crayons. Would the team like to see them?

UNANIMOUSLY: Why not send them to *Nature*?

ANNOUNCER: We'll be back next week.

(*Fade out to strains of Götterdämmerung.*)



EGYPT is taking a cautious approach to one of the world's most spectacular energy projects, the flooding of the Qattara depression, that would create a 14,000 square kilometre salt lake in the Western desert, produce five times as much electricity as the Aswan dam, and lead to the creation of a new industrial centre and a large harbour near El Alamein, the site of a crucial World War II battle.

The project can in fact be traced to World War II, when Friedrich Bassler, a young officer in Rommel's Africa Corps, made a first cursory inspection of the depression which starts some 70 kilometres south of El Alamein, curves west and south for some 300 kilometres and, at its deepest point, reaches 135 metres below sea level. Since then Bassler has become a professor at the University of Darmstadt and an expert in hydraulic engineering. He returned to the depression in the 1960s, made a first study of the project, and came back in 1973, after West Germany and Egypt resumed temporarily interrupted diplomatic relations.

The idea is to cut a 70-kilometre long canal from the Mediterranean to the southernmost tip of the depression (perhaps resorting to the use of nuclear explosives). Water gushing from the sea to the depression would produce electricity, and lead to a number of related developments:

- drilling for oil from floating platforms to exploit deposits said to exist under what are today salty swamps
- developing a petrochemical industry that would put to use salts and minerals accumulating in the artificial lake
- desalinating water and creating an irrigation system for agriculture and animal husbandry
- building a large harbour at the north end of the canal (it would be used, among other things, to export Qattara oil) and a network of roads to service the area.

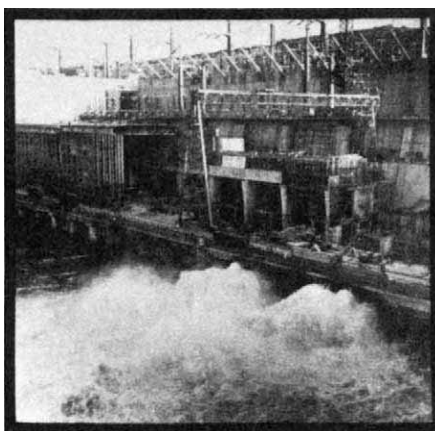
In the past two years, the little talked-about project has progressed considerably. Several West German companies have studied it, Bonn is financing a feasibility study, and work could theoretically start in two years. But the Ministry of Power has decided to convene an international council of Egyptian and foreign experts to study in depth the technical and economic aspects of the project, as well as its possible side effects (it will be the first time man will have created a sizeable salt lake, whose rate of evaporation should be counterbalanced by the constant inflow of water from the Mediterranean).

Although the potential economic benefits are considerable, the possible side effects should not be underestimated. Water desalination is expected to extract much of the

accumulating minerals, but extraction will not keep up with concentration resulting from evaporation, so that the eventuality of a dead sea must be considered. There are, within 100 kilometres of the projected shores, agricultural areas that could be threatened if salt water from the lake reached through fissures into underground fresh water. The result would then be similar to the waterlogging and salinisation which is a major side effect of the Aswan dam.

After the dam the depression?

Egyptians are well aware that the proposed flooding of the Qattara depression in the Western Desert could throw up environmental problems similar to those encountered with the Aswan dam. Alexander Dorozynski reports.



Aswan: unwanted side effects

Some 500,000 acres of desert, irrigated and cultivated in the past 10 years west of the Nile delta are threatened, and salt is even seeping into lower, fertile areas that have been under culture for several thousand years. Irrigation west of the Nile is accomplished by means of a vast network of canals, into which water is pumped through a system installed with the technical and financial help of the Soviet Union.

Groundwater studies had been talked about from the start, but there seemed to be no hurry because most water tables were 20 to 60 metres deep. It turned out, however, that generous irrigation made these tables rise extremely rapidly, in some areas exceeding one centimetre a day (or four metres a year).

Groundwater had a high salt and mineral content, and under the pressure of irrigation water, salt is percolating to the surface, destroying crops, and even seeping into some of the canals, a

few of which have been closed down. A UNESCO-sponsored study showed, in fact, that so much saline groundwater flowed towards the main irrigation canal in the Nubara area 50 kilometres south-west of Alexandria, that the canal now acts as a drain.

The UNESCO study did not come up with a permanent solution, but suggested, in the short term, that the amount of water used should be reduced, and then interception drains whose depth would make them prohibitively costly or wells (also a heavy expenditure, particularly if it is to become a fixed operating cost) should be dug to pump underground water into the sea.

Few people have gone as far as "damning the dam", because there is no denying that its major beneficial objectives have been attained. The High Dam protects the Nile farmer from the vagaries of the river, whether floods or droughts (two years ago, a severe drought would have wrought agricultural havoc if it hadn't been for water released from the 500-kilometre long Lake Nasser.) Up to three and even four crops can now be produced in some areas. And electricity is being generated, the power station working at about half of its capacity of 10 billion kilowatts a year.

Not only are the side effects now being discussed openly, but Sayed Marei (who headed the World Food Conference in Rome last November before becoming president of the National Assembly) has appointed a parliamentary commission to investigate them. The commission's report is expected to be released at any time, but the side effects, in fact, are well known. In addition to waterlogging and salinisation of new agricultural areas, they include the spreading of schistosomiasis through a blood fluke carried by water-borne gastropods (this problem plagues nearly every semi-arid country where the development of irrigation systems has preceded that of hygiene and disease control); the accumulation in the lake of silt that once fertilised the Nile valley and has to be replaced by large amounts of fertilisers; the erosion of the Nile delta, which had kept growing under the accumulation of silt, and where some fresh water lakes and lagoons separated from the sea by narrow land barriers are now threatened with salination; and the drastic reduction in the number of fish off the delta and in the eastern Mediterranean as a result of the disappearance of the silt, an important part of the marine food web (this, however, is in part compensated by the development of fisheries in Lake Nasser). Great caution would obviously be exercised in another pharaonic-scale venture such as the Qattara project. □

international news

AFTER a five-month study, a task force of officials from 14 government agencies in the United States concluded last week that there is "legitimate cause for concern" that fluorocarbons, released into the atmosphere from aerosol spray cans and from discarded refrigerators, are damaging the layer of ozone in the stratosphere which shields the Earth's surface from harsh ultraviolet radiation. As a result, the task force has tentatively recommended that unless fresh evidence soon comes to light to clear up those concerns, fluorocarbons should be banned as aerosol propellants by January 1978, and their use as refrigerants should be tightly controlled.

Even though the task force hedged that recommendation with the caveat that the government should delay taking action for a year to enable a committee of the National Academy of Sciences to complete a thorough study of the matter, the report has already provoked a storm of protest from fluorocarbon manufacturers and from the makers of hair sprays, deodorants sprays and similar cosmetic products.

The task force report was completed almost exactly a year after publication of the first scientific paper suggesting that fluorocarbons (usually referred to by the trade name Freons) may pose a threat to the ozone layer (*Nature*, **249**, 810; 1974). The theory, in short, is that the very properties which make fluorocarbons valuable as aerosol propellants—their inertness and virtual insolubility in water—prevent them from being washed out of the lower atmosphere by rain. They gradually drift up into the stratosphere, where they are broken down by ultraviolet radiation, releasing free chlorine atoms which in turn break down ozone molecules through complex chain reactions.

If that sequence of events is correct, the result would be a reduction in the concentration of ozone in the stratosphere, which in turn would cause an increase in the amount of ultraviolet radiation reaching the Earth's surface. The ultimate consequence would be an increase in the incidence of skin cancer, and possible biological damage to plants and animals.

The theory cannot be supported by direct measurements of ozone concentration in the stratosphere because available measuring techniques are too insensitive to detect relatively small changes in the ozone layer. Nevertheless, the task force notes that the

US task force wants ban on aerosol gases

by Colin Norman, Washington

theory has been studied independently by many scientists and "thus far, the validity of the theory and the predicted amounts of ozone reduction have not been seriously challenged".

In short, the task force concludes that "the best estimates" are that fluorocarbons already released into the atmosphere may have reduced ozone concentration by between 0.5% and 1.0% and even if no more were released, the average ozone concentration would continue to drop as fluorocarbons now present in the lower atmosphere gradually work their way into the stratosphere. The total depletion from already-released fluorocarbons may eventually amount to between 1.3% and 3.0%, the task force suggests. Moreover, the report cites calculations predicting that the ozone layer will eventually be depleted by about 7% if fluorocarbon production is maintained at the 1972 level.

Those calculations could, however, be knocked for six by two possible factors. First, if a natural sink for chlorine atoms were discovered in the stratosphere, the postulated chain reactions leading to the breakdown of ozone would not take place. And second, if chlorine from natural sources were detected in the stratosphere in amounts which dwarf the predicted levels from man-made sources, then there would clearly be something wrong with the theories about how the ozone balance is maintained in the stratosphere. The task force therefore recommends that research be stepped up to determine whether either of those factors is present.

But, in the absence of data which would either confirm or invalidate the theory, the task force believes that the federal government should err on the side of caution and put a stop to release of fluorocarbons into the atmosphere.

What effect would such a decision have on industry? Total fluorocarbon production in 1973 in the United States and western Europe amounted to about 1.7 billion pounds, and about half the

production and use occurred in the United States. US production has been doubling about every six years, although there have recently been signs of a slackening in the growth rate. About half the US use is accounted for by aerosol sprays, such as hair sprays, deodorants, anti-perspirants and so on, and another 28% is used as refrigerants.

The task force acknowledges that although alternative propellants exist for some products, there are now no alternatives for others. Nevertheless, the report points out that "the cosmetic industry will continue to manufacture cosmetics" — it will simply switch from aerosols sprays to other delivery systems, such as roll-on deodorants. Substitutes for fluorocarbon refrigerants may, however, pose more of a problem. But the task force suggests that instead of banning such refrigerants outright, "the problem might be partially solved through reduction of leakage and recovery of the fluorocarbons for recycling at the time of eventual disposal".

One important task force recommendation is that all aerosols using fluorocarbon propellants should be so labelled. With increased consumer awareness of the possibility that fluorocarbons may damage the ozone layer, a switch to other products could be anticipated.

It should be noted that there are several factors which may prevent the task force's recommendations from being adopted. For one thing, responsibility for regulating the various products containing fluorocarbons is now divided among a variety of federal agencies, which would make concerted action rather difficult. And for another, the fluorocarbon manufacturing industry and the cosmetics industry would be sure to fight attempted controls in court, which could result in long delays in the regulatory process.

Finally, the task force points out that since the effects of a reduction in the ozone layer would not respect national boundaries, international action would be desirable. "It is particularly important", the task force states, "that these conclusions and recommendations be made known to other countries producing and using fluorocarbons and that concerted international action be sought through appropriate international organisations and bilateral channels". □

Russians have space link in mind

from Vera Rich

As the final preparations are made for the joint Apollo-Soyuz mission in July 1975, the Soviet space programme is continuing actively along its own individual course. In spite of the avowed cooperation on the international mission—with mutual understanding guaranteed by Russians speaking English and Americans speaking Russian, and a planned atmospheric pressure for the linked craft of 10 pounds per square inch (intermediate between the standard Soyuz atmospheric pressure and the Apollo pressure of 5 pounds per square inch pure oxygen), the Soviet planners, after last year's setbacks, seem to display a pardonable keenness to show their American colleagues what they can, if necessary, do alone.

Accordingly, the Soyuz 18-Salyut 4 mission, manned by two experienced cosmonauts Petr I. Klimuk and Vitalii I. Sevast'yanov, has embarked on a wide ranging programme of experiments, ranging from a complete testing of the Soyuz on-board systems to the

growing of vegetables in conditions of weightlessness, photographic and spectrographic observations of the Earth's surface (including the track of the Soviet Union's latest prestige engineering project—the Baikal-Amur railway), the recycling of atmospheric condensation, and "observations of the Sun, planets and stars in various ranges of the electromagnetic spectrum". Although Soviet press coverage is not noted for superlatives, the coverage of the current mission seems to have been deliberately low-key; the tone is one of business as usual, rather than heroic achievement. Although it is stressed that this mission is a routine part of the Soyuz-Salyut programme, it would surely be impossible for the Soviet space planners not to bear in mind the significance of the forthcoming international mission.

● The unmanned sector of the Soviet space programme is also proceeding well. In addition to the routine Kosmos launches, an unmanned lunar orbiter (Luna 22) was launched on May 29 and, on June 5, the French minisatellite MAS-2 was launched from the same carrier as the latest Soviet communications satellite of the Molniya 1 series. Most fortunately of all, the occurrence of a Venus window made it possible for

the latest Soviet Venus probe, Venera 9, to be launched on June 9. The plans for this mission have been announced only in the most general terms: "to continue scientific observations of the planet Venus and its ambient space" and, en route, "to investigate the physical characteristics of interplanetary space, in particular, to make measurements of the characteristics of the interplanetary magnetic fields, the solar wind and ultraviolet radiation". The successful soft landing of the previous Venus probe, Venera 8, would suggest, however, that a similar landing may be attempted on this occasion, particularly as the probe is scheduled to arrive in the vicinity of the planet in October, coinciding neatly with the postponed 250th anniversary celebrations of the Soviet Academy of Sciences.

● A new conservation programme for the Carpathians and sub-Carpathia is being introduced by the Academy of Sciences of the Ukrainian SSR, to restore the ecological balance destroyed by long term deforestation and "un-systematic use" of the alpine pastures. The project aims not only to extend the forest cover of the region by some 10–15% but also to select the species planted in order to ensure maximum use of the natural precipitation which, at some 1,600 mm a year, is among the highest in central Europe. Surveys have indicated that stands of different species retain precipitation to different extents—whereas beech will retain some 25%, Carpathian fir will retain up to 37% of the incident precipitation per hectare. Under the new scheme, the Carpathian forests, for centuries a major source of timber, and, with sheep-rearing, the staple industry of the area, are to be considered to be "significant hydrologically, rather than as raw materials".

● The new Soviet currency regulations, to be introduced in January 1976, are expected to strike particularly at the Jewish 'refusnik' scientists, dismissed from their academic posts after applying for exit visas for Israel. Under the new regulations, an additional state charge will be levied on all money transfers to the Soviet Union from abroad, in addition to the existing State Bank duty of 35%. Although this surcharge will, of course, affect all refusniks, who are often almost totally dependent on financial support from friends and relatives abroad, it is liable to be a special burden upon scientists, whose chances of emigration are considerably less than those of the average worker, and who, as members of the intelligentsia, are more likely to be selected as an example to discourage the rank-and-file of would-be emigrants. □

THE wrong professionals are leading the field of population science in the United States, according to the president of the World Population Society. Dr Charles M. Cargille, who is also assistant dean at the University of North Dakota's medical school thinks that demographers and obstetricians should be replaced by engineers—and he has proposed as much to the National Research Council.

Obstetricians make money delivering babies, he reasons, and therefore there would seem to be a clear conflict of interest when they turn their attentions to population science. And demographers, who have long been the real professionals in population science, gave no hint at their recent annual meeting that there might be a population problem.

Dr Cargille recently told the Canadian Association for the Club of Rome that, a real conceptual framework was lacking for population science, which is a discipline the objective of which is problem-solving. According to Dr Cargille demographers leave it to the politicians and have no idea what to do about the population problem. An effective solution is unlikely because of the inadequacy of the population "establishment", he said.

Not a single population project is to be found intramurally in the National

Institutes of Health, the institutional home of population science, and he proposed that responsibility for it should be transferred to a new agency. In addition, budgets for population science have been cut and its principal leaders have died, without new ones emerging.

But the chief reason for the deficiency is that 72–78% of the appropriate US funds have been siphoned off into basic reproductive biology since 1968. Dr Cargille called this a "mistake in public policy".

Population science, he considers, needs multi-billion dollar funding, and should be addressing itself to such questions as: What is the global population capacity? How will the collapse of populations from famines feed back to developed countries? How can we achieve stable population growth? Where does critical pollution occur?

Dr Cargille's sombre approach contrasted somewhat with that of Dr Leonard H. Shebeski, dean of the faculty of agriculture at the University of Manitoba, who told the same meeting that the present world food output could be tripled to provide food enough for at least 10,000 million people. But Dr Shebeski saw that there could be political objections to the full development of arable lands.

from David Spurgeon, Ottawa

BURIED deep in the report on domestic activities conducted by the Central Intelligence Agency, published last week by a special commission headed by Vice-president Rockefeller, is an account of an extraordinary and "clearly illegal" programme carried out by the agency's Directorate of Science and Technology. It involved giving psychotropic drugs, such as LSD, to unsuspecting people as part of an effort "to counter the use of behaviour-influencing drugs clandestinely administered by an enemy."

At least one person who was fed LSD without his knowledge "developed serious side effects" and later jumped to his death from a tenth floor hospital window. And there were also several cases in which victims became ill for hours or days after unknowingly taking the CIA's drugs.

The drug testing programme, the Rockefeller report says, "was part of a much larger CIA programme to study possible means for controlling human behaviour. Other studies explored the effects of radiation, electric-shock, psychology, psychiatry, sociology and harassment substances".

The full extent of the programme is not known, however, because all the records were destroyed in 1973, "including a total of 152 files", the report states. Moreover, CIA officials concerned with the programme couldn't be interviewed by the Rockefeller Commission because they were either dead or out of the country. Those officials, according to one news account, include the former head of the CIA's Directorate of Science and Technology, who left the United States in 1973 and is now living abroad.

The CIA's interest in the use of drugs in intelligence operations first surfaced in the late 1940s, prompted by reports that the Soviet Union was experimenting with behaviour-inducing drugs and by speculation that confessions introduced in trials in the Soviet Union had been obtained by the use of drugs. But testing did not begin until 1953, the report states.

First, a few tests with LSD were run on volunteers, but in 1955, "tests were begun on unsuspecting subjects in normal social conditions". Testing began on the West Coast, and a similar programme was started on the East Coast in 1961.

The report goes on to note that administration of dangerous substances to unsuspecting people was halted in 1963, and all drug testing was then carried out on volunteers, chiefly prisoners. The entire operation was finally terminated in 1967.

The incident which resulted in a victim throwing himself out of a hos-

pital window took place in 1953. "LSD was administered to an employee of the Department of the Army without his knowledge while he was attending a meeting with CIA personnel working on the drug project", the report states. Although he had participated in discussions "where the testing of drugs on unsuspecting people had been agreed to in principle . . . this individual was not made aware that he had been given LSD until about 20 minutes after it had been administered".

Washington seen

by Colin Norman, Washington



The two CIA employees responsible for the incident were merely reprimanded by the CIA Director, the report states, which indicates that the drug programme was at least brought to the attention of the agency's top brass.

The Rockefeller Commission also notes that the CIA Directorate of Science and Technology develops equipment for monitoring conversations, and the report reveals that such equipment has been tested in the United States by clandestine monitoring of private conversations. Some conversations were recorded, the report states, "but the recordings were kept only until the testing was concluded". The Commission recommends that "testing of equipment for monitoring conversations should not involve unsuspecting persons living within the United States".

● According to two briefings given by Vice-president Nelson Rockefeller to Congressional committees last week, the science policy office which President Ford wants to establish in the White House (see *Nature*, 255, 438; 1975) will have broad responsibilities, and it will closely resemble the Office of Science and Technology (OST), which President Nixon scrapped in 1973.

Most important, Rockefeller informed Senator Kennedy's NSF subcommittee and the House Committee on Science and Technology that the proposed office will be expected to advise the President on military research and development. That responsibility was specifically excluded from

the functions assigned to Dr Guyford Stever, the Director of the National Science Foundation, when Nixon conferred upon him the title of Science Adviser in 1973, following the destruction of OST. The lack of influential outside scientific scrutiny of the Defense Department's programmes is regarded as a major deficiency in the present science policy apparatus.

According to Rockefeller, the office—which would bear the title of the Office of Science and Technology Policy—would consist of up to 15 people, headed by a director and a deputy director, and it would be equipped with a budget of about \$1.5 million a year. By comparison, the old OST had a staff of about 25 and a budget of about \$2 million, while the science advisory apparatus now operating in the National Science Foundation has a budget of about \$6 million.

Asked in an interview last week what would become of NSF's policy apparatus when the advisory functions are shifted back to the White House, Stever said that he expects NSF to retain a small office, chiefly for longer-term analyses, to assist the White House.

Noting the similarity between the proposed White House office and the old OST, Stever said that there is nothing inherently wrong with the structure, but it just didn't work well in the Nixon White House. The Ford Administration, he suggested, has shown more interest in science advice and there is no reason why the arrangement could not be made to work.

● The National Academy of Sciences has been asked to undertake a thorough review of the role that nuclear power should play in long-term energy supply in the United States. The study, which will be carried out for the Energy Research and Development Administration (ERDA), will be announced when the contract is signed and a committee is appointed. Although it will take up to two years to complete, the study could play an important role in the Congressional debate on ERDA's budget, which is expected to take place in the next few weeks.

An important part of the study will be an assessment of the need for a fast breeder reactor in the United States, and Congressional opponents of the breeder programme are expected to attempt to remove some funds for the programme from ERDA's budget until the Academy's verdict is in. Although there has recently been an increase in opposition to nuclear power among members of Congress, the strength of that opposition has yet to be tested in a vote. This year's debate on the ERDA budget could provide just such a test.

B RITISH university teachers this month got their pay award from the government at the hands of arbitrators, called off their campaign of non-cooperation in the marking of examinations and so ended the short lived drama of academic militancy. The arbitration tribunal, whose findings are likely to be accepted by the government, gave lecturers a starting salary of £2,778, senior lecturers £5,838 and professors an average of £8,884. These figures will attract a separate cost of living element that could average 20%. The award represents the 'catching up' exercise that has been at the centre of the dispute between government and university academic staff for the past year.

But the euphoria over a settlement that, with the second element yet to be negotiated, could give university teachers a salary increase of nearly 50% by comparison with October 1974, should not be allowed to conceal irreversible changes in the relationship of academics and government. Not only have academics woken from their political slumber and taken direct action against their employers, but for the first time they have identified their employers clearly as the government. The paraphernalia of the 'buffer mechanism' of the University Grants Committee and university authorities has become a sideshow.

The history of the claim that led to the first strike ever attempted by academics goes back to early 1974. The Association of University Teachers believed that the settlement into which it was somehow cajoled during that year put it firmly behind all other comparable groups as from October 1973. The government rejected this argument and made it clear that it would negotiate a settlement for the year 1974-75 that would run from October 1975 and no sooner. Anything else, the Department of Education and Science has consistently argued, would be in breach of the social contract.

This picture of leads and lags in salary negotiations, coupled with apparently growing differentials between university people and fellow professionals in the Civil Service and in the rest of the higher education sector was darkened in the past 18 months by general reductions of government spending on the universities. There have been no absolute cutbacks, but a feeling of gloom and immobility grew, convincing many academics that the universities were a target for educational iconoclasts within the Cabinet.

During the summer of 1974, the Association of University Teachers (AUT), which now represents about

25,000 academics, settled a salary scale to begin in October. The revision of that scale, which gave a junior lecturer a starting salary of £2,118, a senior lecturer £4,707 and an 'average' professor £7,125 has just been completed. The scale for October 1974 emerged at the end of two sets of negotiations and after the deliberations of two separate committees. University teachers negotiate first with the university authorities and then, together with them, with the Department of Education and Science.

The first agreement in 1974 was made under the old Conservative

University teachers show their teeth

from David Walker

government's incomes policy, given a few extra months of life by its Labour successor. At the end of statutory controls in July the agreement was changed by a few per cent. By November 1974 the AUT had begun further negotiations with the university authorities on a claim for an 18% increase in the October scales. At Christmas the claim passed to the government.

That AUT claim held up the Civil Service as the main reference group for academics but the publication on Christmas Eve of a report by a committee under Lord Houghton on the salaries of school and further education teachers immediately set up polytechnic lecturers as rivals and envied competitors in the salary race. The report of the Houghton Committee will probably come to be seen as one of the most divisive education documents ever published. In recent weeks university teachers have evinced suspicion of the quality of work done in polytechnics, and near hatred of a government that could apparently favour degree level work in such institutions at the universities' expense.

The campaign by university teachers on what was now seen as deep anomalies in their pay gathered steam in the early months of 1975. In February the Committee of Vice-Chancellors and Principals expressed "very great concern" about recruitment and standards because of salary differentials. Allowing for adjustment between scales, a polytechnic lecturer of comparable age could be earning—in May—£1,000 more than a university man. There was a difference of £296 between the salary of a senior lecturer in a university and of his polytechnic equivalent.

Meanwhile the Labour government produced its own version of the cuts in public expenditure made by Mr

Anthony Barber during the last days of the Conservative government in 1973. Many academics came to the conclusion that while cuts were being made all round with the universities suffering as a consequence the polytechnics were being buoyed up by grants for accommodation for students, and not least by the Houghton settlement.

Against this background Professor William Wallace of the New University of Ulster, President of the AUT, called the polytechnics "overvalued" and threats began to be made concerning operation of the Council for National Academic Awards, staffed in the main by university academics, which validates the degree work of polytechnics and other further education colleges.

The campaign came to a head on May 6 when the AUT called a national "day of action" during which well attended meetings were held in most universities to protest about "discrimination" by the government. At the end of May the academics took their irritation to a council meeting of the AUT addressed by Lord Crowther-Hunt, the minister responsible for Higher Education.

By that time heated negotiations between the teachers and the government had ended only in agreement to go to arbitration. To keep up the pressure the AUT decided to recommend members to withhold examination results, a measure which would take full effect only later, in July.

The arbitrators have been generous. Lecturers will get a maximum of £6,050 and the minimum for professors goes up to £7,501. To which, of course, has to be added the cost of living element. This award is marginally above what the Department of Education offered the university teachers in May. Their claim for a separate element to take care of what they called "falling behind" during 1973-74 has now effectively been relinquished.

But what remains are deep feelings of resentment among many academics about what has been called the injustice of government policy. During the months of agitated negotiations, however, government policy has changed: Lord Crowther-Hunt has recently given a series of speeches which shows that the universities have lost most of their "special" attributes in the eyes not just of the Labour government but of any government that has to trim public expenditure on education. Just as university teachers plan strikes in the same way as manual workers, so universities are now irrefutably part of a system of higher education within which polytechnics and further education might be favoured.

news and views

Megalithic astronomy: a prehistorian's view

from Andrew Fleming

THE suggestion that the Neolithic and Bronze Age peoples of north-west Europe may have been brilliant astronomers and geometers has been met with stupefaction by prehistorians, who faced the recent spate of papers on the subject in total disarray. Most have not felt competent to judge the statistical and astronomical significance of the work of Alexander Thom and others. Professor R. J. C. Atkinson has now come to the conclusion, however, that it is more reasonable to change his model of European prehistory than to dismiss the results as due to chance (*J. History Astron.*, 6). It seems likely, however, that any model of European prehistoric processes which changed to accommodate Thom's ideas would itself strain credulity.

What is at issue is not the intelligence of prehistoric peoples, nor their capacity for organisation, nor yet the possibility that they were interested in the movements of the heavenly bodies. Indeed it is probable that they were so interested. There is good archaeological evidence for a Sun-cult in some areas. In north-west Europe it would be essential for early farmers to identify abnormal seasonal weather patterns, especially in the Highland Zone where so many of Thom's sites are located. There is some archaeological evidence for regional gatherings, which would have to be synchronised in some way; it is unlikely that ceremonials involving advance preparations of various sorts and careful scheduling of agricultural operations would have been summoned by casual smoke-signals at a few days' notice. Certainly lowlier species than man have annually synchronised behaviour patterns, the commencement of the breeding season being accompanied by individual and communal displays and a high level of social activity.

European prehistoric peoples also orientated their monuments roughly, displaying at least knowledge of the Sun's behaviour in relation to the local sky line. Indeed in the case of New Grange, the great third millennium passage grave in eastern Ireland, the midwinter Sun shines through the

'roofbox' and illuminates the chamber, which is at the end of a 15-m long passage—a very clever piece of megalithic design. Nor is this kind of habit confined to man; the satin bower-bird of Australia, for instance, orientates its 'bower' within 30° of 360°, which apparently has the effect of allowing the male to display while keeping the female in sight and without staring into the Sun she too can watch the impressive display in comfort.

In a recent article about the *Grand Menhir Brisé* at Er Grah in Brittany, Thom says "No one who sees Er Grah can fail to be impressed, or to ask the reason for its being there. Many explanations have been advanced but they all fail to account for the sheer size of the stone or indeed for its position." As a prehistorian I too am impressed by ceremonial monuments—including mediaeval cathedrals, follies, war memorials and so on—but this is simply a tribute to the visual effect of these socially integrative devices. Any astronomical 'explanation' will have to include an account of their role as monuments. Clearly the most convincing cases will be those where monumental design and significant alignment are integrated; New Grange and Stonehenge are good examples, and in south-west Ireland the main axes of the stone circles are clearly indicated. Lines which pass from the centre of truly circular structures through outlying standing stones also count as clearly indicated directions; so do the axes of Class II henge sites. One would expect the initial demonstration to have been established from these unambiguous types of site. Unfortunately, prehistorians are now faced with all manner of claimed astronomical directions, involving rugged skylines, broken, recumbent menhirs, excavated post holes, stone alignments, cairns and barrows, unexplained humps and bumps, and even in one case straight, presumably modern tracks. Standing stones can be interpreted as general pointers or precise indicators; at various times their tops, lower portions or flattened sides can be considered as significant.

In short, there is no standard type of

observatory—and this in an area embracing a zone from Brittany to the Orkneys—where the standard unit of length suggested by Thom, the 'megalithic yard' varied by less than one two-hundredth of an inch! This heterogeneity has to be properly explained, a task which has not been attempted so far.

We are not told how far some astronomical alignments may have been marked out at a later stage in a site's development, or how far the very position and layout of the site were determined by a set of previous astronomical observations. It would be interesting to know how many sites have no apparent alignments, and whether these conform to any regional or taxonomic pattern. If we are to believe that the role of these sites as observatories preceded and was more important than their ceremonial role, we also need to be shown how they might have operated within their societies. Prehistorians have never been told how far these sites could be used by a group of worshippers similar to the much-mocked modern Druids at Stonehenge and how far they are simply observatories used by the priests for calendrical or eclipse-predicting purposes. Certainly Thom at times suggests that eclipse prediction and 'special effects' may have been used to impress the populace. It is likely that in societies at this technological level ceremonials would have played an important role, but it is very hard to believe that the sort of mass manipulation envisaged by the 'crafty priests' school of thought would have been possible, necessary, or effective in rural Scottish groups. The functions discharged by ceremonial, and the need to have recognised ritual sites and other symbolic points in the landscape, would certainly have taken precedence over the painstaking manoeuvres necessary for the setting up of observatories.

Until some reconciliation can be achieved between the sites as ceremonial monuments and as complicated solutions to astronomical puzzles, it will take more than clever statistical arguments to convince prehistorians that more than a handful of the present claims can be justified. □

Ia antigens and F_c receptors

from Robert S. Kerbel

IDEAS come and go, certainly, but the rapidity with which they occasionally do so can leave one a little breathless. Barely has the latest, most fashionable theory been digested before it is threatened with obsolescence.

Take the case of Ia antigens and F_c receptors: the former are alloantigens determined by the I (immune response) region of the mouse major histocompatibility (H-2) complex while the latter are membrane-associated receptors which recognise the F_c portion of certain immunoglobulin molecules. F_c receptors are found on a wide variety of cells including B lymphocytes, activated

T cells, macrophages, neutrophils and eosinophils. The function of both these entities is unclear and currently the subject of interest but they have been implicated in a wide spectrum of immune phenomena including genetic control of immune responses, mechanisms of cell cooperation, and regulation of immune responses. It was therefore a finding of considerable potential importance when Dickler and Sachs (*J. exp. Med.*, **140**, 779; 1974) reported evidence for an identity or close association of the F_c receptor and Ia antigens on B lymphocytes.

The evidence consisted primarily of inhibition experiments in which the uptake of whole heat-aggregated immunoglobulin (Agg-Ig) molecules by mouse B cells—one way of apparently detecting F_c receptors—could be significantly inhibited by preincubating the cells with specific anti-Ia sera, but not with iso-antisera which reacted with the antigens determined by the K or D regions of the H-2 complex, nor with a heterologous anti-mouse Ig serum which reacted with Ig-bearing mouse B cells. Thus, there seemed to be some sort of unique relationship between alloantigens controlled by the I region of the H2 complex and the F_c receptor.

This conclusion has now been challenged by the findings reported by Schirrmacher, Halloran, and David (*J. exp. Med.*, **141**, 1201; 1975). These investigators essentially repeated Dickler and Sachs's experiments, but used two alternative assays to detect F_c receptor-bearing cells. One of these, called F_c or EA rosette formation, detects F_c-receptor bearing cells by virtue of the cells forming clusters or 'rosettes' with IgG antibody-coated erythrocytes. When Schirrmacher *et al.* preincubated their B cells with anti-Ia sera, F_c rosette formation was markedly diminished, in agreement with Dickler and Sachs. But when the same procedure was repeated using a variety of other antisera, all of which react with B cells, including anti-

Ly4.2, anti-MBLA, and anti-Ig, inhibition of rosette formation also occurred. Anti-T cell sera did not effect such inhibition. Furthermore, the authors cited further experiments in which anti-H2D or anti-H2K sera caused significant inhibition. The authors also showed that F(ab)₂ fragments of some of the various antisera preparations possessed similar inhibiting capacities to the intact molecules, which proved that the observed inhibition using intact antisera was not due to the formation of third party immune complexes pre-empting F_c receptors, as been observed in other assay systems detecting F_c receptors (Halloran and Festenstein, *Nature*, **250**, 52; 1974; Basten *et al.*, *J. exp. Med.*, **141**, 547; 1975).

On the face of it, these results certainly provide no support for the view that a unique association exists between Ia antigens and F_c receptors, although, in fairness, they do not formally disprove the notion either. For example, if it can be shown that 'capping' of Ia antigens also leads to co-capping of F_c receptors whereas capping of Ly4.2 or MBLA antigens does not, then it could still be argued that a unique association between Ia antigens and F_c receptors does indeed exist.

For the moment, the obvious question is: why the differences in results? An equally obvious answer would be to implicate the different assay systems used. There have been a number of findings lately which seem to make it clear that the F_c rosette test and the Agg-Ig binding assay may not be detecting the same receptor, or the same cell(s). Indeed Froland, Natvig, and Michaelsen (*Scand. J. Immun.*, **3**, 375; 1974) have recently claimed that the binding of aggregated IgG by human B lymphocytes can occur independent of F_c receptors. Conversely EA rosette formation required F_c receptors, but—at least in their experiments—the rosette-forming cells did not seem to be classical Ig-bearing B cells.

With regard to the conflicting results obtained when using these two different assay systems, it should also be noted that Schirrmacher *et al.* used an additional, functional, test system to detect F_c receptors and, once again, found no evidence for a unique association between Ia antigens and F_c receptors. The system involves the use of antibody-coated chicken erythrocytes as target cells in a cytotoxic assay in which normal spleen cells attack the target cells by virtue of F_c receptors on the killer cells (K cells) interacting with the membrane-bound antibody molecules. When antibody molecules with specificity for the K cell or any other cell found in normal spleen, are added to the system, inhibition of cytotoxicity is observed as a result of pre-emption

of the F_c receptor on the K cell by the antibody-coated spleen cells (Halloran, Schirrmacher, and Festenstein, *J. exp. Med.*, **140**, 1348; 1974). This assay, called 'cytotoxicity inhibition assay' or a timely CIA for short (none of the authors is American), demonstrated that F_c receptor-bearing K cell activity was inhibited by all of the sera mentioned above, including anti-T cell sera. More importantly F(ab')₂ fragments of the antisera—including anti-Ia—did not cause any inhibition. Once again, if the F_c receptor and Ia antigens are identical or closely associated, one might have expected some inhibition of K cell activity by the addition of F(ab')₂ fragment of the anti-Ia serum.

It therefore seems prudent at this time to exercise restraint before making any categorical assumptions about the association of F_c receptors and Ia antigens.

Organisation and disorder in membranes

from J. C. Metcalfe

SINCE Gorter and Grendel introduced the bilayer concept in 1925, the many speculative models of membrane structure have not led to any precise insight into how structure is related to function. At present, the fluid-mosaic model, popularised by Singer and Nicholson, has the merit of emphasising the balance between the structural organisation and the fluid disorder of membrane lipids and proteins, which are both established on an experimental basis. In different membranes this balance ranges from the extremely fluid disk membranes of rod outer segments, in which rhodopsin and the lipids diffuse laterally at rates of up to a micrometre a second, to the very rigid two-dimensional lattice of 'bacteriorhodopsin' which is the only protein in the purple membrane fragments of halobacteria. This contrast is especially striking because both proteins use the same retinal chromophore to transform light into a biochemical signal.

The IUB-IUPAC Symposium in Tehran (5-7 May) on "The Structural Basis of Membrane Function" provided some striking examples of the underlying tension between structural order and fluidity in modulating the many biochemical functions which can coexist in a single membrane. The purple membrane, which combines extreme order and simplicity of composition, provides an exceptional opportunity for a complete structural and functional analysis. The bacteriorhodopsin functions as a light-driven proton pump and W. Stoeckenius (University of California) reported that at least four inter-

mediate states of the bacteriorhodopsin can be detected by laser resonance Raman spectroscopy. These are involved in the cyclical association and dissociation of protons with the retinylidene-lysine linkage, a Schiff base which seems to crank the proton pump. Stoekenius also illustrated an exciting foretaste of the work of R. Henderson and P. N. T. Unwin (MRC, Cambridge) who have made a brilliant application of new electron microscopy techniques to provide a 7 Å electron density profile of the bacteriorhodopsin lattice, projected on to the plane of the membrane. The projection indicates a number of helices perpendicular to the plane of the membrane and apparently spanning the bilayer, and is surely a sign of much more to come. Taken together these studies suggest that the purple membrane will be the first for which a detailed structural model will accommodate a specific functional protein.

Another membrane fraction enriched with a lattice-like array of protein molecules is obtained from the excitable membrane of *Electrophorus electricus*. New evidence was presented by J.-P. Changeux (Pasteur Institute, Paris) that the nicotinic receptor lattice can be transformed into a desensitised state by calcium, local anaesthetics, and changes in the lipid environment of the protein, in which it has a much higher affinity for acetylcholine than in the functional state. R. W. Eastabrook (University of Texas, Dallas) also showed that the P450 cytochrome molecules in microsomal membranes form clusters, which exist as a persistent entity with a fluid lipid bilayer. A working hypothesis for the activation of adenyl cyclase by cholera toxin (P. Cuatrecasas, Johns Hopkins University), involves the formation of an inactive complex of toxin with the ganglioside receptor, which subsequently migrates laterally in the plane of the membrane to form patches of the complex. It is then transformed into an active species by dissociation of the hydrophobic subunit of the toxin into the bilayer, which directly activates the cyclase (virtually irreversibly).

This raises the more general question of the trans-membrane coupling of hormone receptors on the outer surface of the membrane with the adenyl cyclase moiety on the inner surface: do they migrate independently in the two halves of the bilayer when uncoupled until the hormone provides the signal for a locking interaction, or is the receptor always associated with the enzyme as a multicomponent entity? This is clearly susceptible to biochemical analysis, but the difficulty in placing constraints on molecular models from a purely functional analysis of membrane phenomena was well



A hundred years ago

A NEW steering balloon by Smither is being exhibited, suspended in the middle of the Alcazar in Paris. The measurement is only 6,000 cubic feet, but the balloon is so light, that when filled with pure hydrogen it must float. A considerable sum of money has been invested in it, and great ability has been displayed in the construction. Although no practicable result in open air may be hoped for, it is a wonderful piece of clockwork.
from *Nature*, 12, 153; June 24, 1875

illustrated by R. D. Keynes (University of Cambridge), who described some elegant experiments on the gating current in squid axons. The initial opening of the sodium channels in the conduction of the action potential is brought about by the movement in the electric field of a group of charged gating particles of which there are three per channel, and the subsequent relaxation to the impermeable state apparently occurs by a time-dependent conformation change. The importance of direct biochemical evidence for structural properties of membrane proteins was emphasised in a review of the transport of metabolites by vesicles from *E. coli* by H. R. Kabach (Roche Institute, New Jersey) which included the striking result that the *lac* carrier protein is inaccessible to fluorescent inhibitors of lactose transport in the external solvent in the absence of energy coupling, but on generation of a membrane potential, binding of dansyl galactosides is observed. Alternatively binding can be induced by lactose efflux through the carrier and down a concentration gradient.

In addition to these features of the structure and function of the membrane proteins, the asymmetric distribution of the lipids in the bilayer is now firmly established. The biochemical approach of using pure phospholipase to digest selectively the lipids in the inner and outer halves of the erythrocyte bilayer has worked surprisingly well in Van Deenen's group (University of Utrecht), and there is a good qualitative agreement with the previous chemical labelling techniques of Bretscher. It seems that in many plasma membranes the lipids with choline headgroups are predominantly located in the outer half of the bilayer. Wisniewski *et al.* (*Proc. natn. Acad. Sci. U.S.A.*, 71, 4381; 1974) have recently suggested that transport proteins in

mammalian cytoplasmic membranes sense independent lateral phase separations of lipids in the inner and outer halves of the bilayer, which presumably requires an asymmetric lipid distribution.

To the prejudiced outsider, the mitochondrion has long seemed like the Boot Hill of the membrane field. But as the plethora of undefined mitochondrial factors, described in another context by Bangham as "more like a culinary extravaganza than a scientific experiment", have given way to biochemical studies of isolated proteins and functional complexes reconstituted from defined components, the functional relationships between components can be defined much more precisely. A powerful assault on the function and disposition of the seven polypeptides of cytochrome oxidase from yeast by immunological and genetic techniques was summarised by G. Schatz (Biozentrum, Basel). The three largest polypeptides are synthesised in the mitochondrion, and two of these are hydrophobic and relatively inaccessible in the 'core' of the enzyme. But the catalytic activity of the enzyme requires subunits which are synthesised in both the mitochondrion and the cytoplasm, which presents a very interesting problem in the biosynthetic integration of the complex structure. M. Klingenberg (Universität München) described his elegant work on the purification of the ADP/ATP carrier in mitochondria, using inhibitor protection to hold together the two subunits of molecular weight 30,000. In the intact membrane it was shown that the protein functions as a gated pore rather than a rotating mobile carrier and is structurally asymmetric.

The relationships between ion fluxes and solute transport which can be generated by mitochondria are sufficiently complicated that as G. F. Azzone (University of Padua) remarked after discussion of cation extrusion processes, it is still impossible to be dogmatic about opposing theories of the coupling between metabolism and transport. Nevertheless the groundswell has been moving strongly in favour of Mitchell's chemiosmotic hypothesis for several years, and in other systems the evidence seems relatively clear-cut. M. Avron (Weizmann Institute, Rehovot) discussed the light-dependent formation of ATP by chloroplasts, and showed that photophosphorylation is a linear function of the proton concentration gradient within a small range of pH across the membrane, and that no phosphorylation can be observed below a critical threshold. This explains the lag in the time course of phosphorylation at very low light intensities, but surprisingly, neither the threshold nor the linear dependence varies signifi-

cantly with the phosphate potential.

Even an account as selective and idiosyncratic as this cannot end without acknowledging the scientific and organisational contribution of Dr J. Hatefi (Scripps Clinic and Research Foundation, La Jolla) and the committee of the University of Tehran. I hope that the interaction and contacts with Iranian biochemists will help to overcome the isolation which distance can still impose on the physical periphery of scientific communities. This would at least partially repay our hosts for a unique meeting.

Tropical forest genetics

from Ken Eldridge

"Variation, Breeding and Conservation of Tropical Forest Trees" was the title of the international symposium conducted jointly by the Linnean Society of London, the International Union of Forestry Research Organisations (IUFRO) and the Commonwealth Forestry Institute, Oxford, 17-19 April. The meeting continued in Nigeria and Ghana as a IUFRO field workshop until 2 May.

"A FORESTER is a man who cuts trees down, wishes he hadn't and then tries to put them back", suggested K. A. Longman, of the Institute of Terrestrial Ecology, Edinburgh. Much of the discussion at the symposium centred on the loss of irreplaceable genetic resources of tropical forest trees, denying future generations the opportunity to find new crop plants for wood, food, fibre and medicines.

The evergreen forests of the wet tropics comprise 20% of the area of the world's remaining forest and carry 50% of the wood, a major natural resource which should be renewable. In fact it is not being renewed and its use, as J. F. Hughes, of Oxford University, said, is "generally inefficient and often potentially disastrous".

Several speakers emphasised the numerous kinds of breeding system and patterns of genetic variability in tropical forest trees. Some of the Meliaceae grow as rare individuals a long way apart increasing the chance of selfing; *Triplochiton scleroxylon* grows in small but widely separated groups, favouring the inbreeding of relatives; and tropical pines are in nearly pure stands, favouring outcrossing. Pollination can be by a multitude of insects, birds, bats, small mammals, wind, or various combinations. While some rain-forest trees flower and seed almost continuously, others seed heavily only at intervals of several years.

T. Hedegart reported the work of the Thai-Danish Teak Improvement Centre which has the most advanced study of the breeding system of a tropical forest tree. The small flowers (6-8 mm diameter) have a one-day cycle and are receptive for only a few hours about mid-day. Experiments with hand pollination showed a high degree (96-100%) of self incompatibility. When insect visitors were caught and washed it was found that two species of bee carried most teak pollen.

Generalisations are not appropriate for highly variable characteristics of breeding systems. For this reason K. S. Bawa, of the University of Massachusetts, Boston, drew attention to the folly of uncritically applying genetic information from the relatively well-known northern conifers to planning the conservation of genetic resources and genetic improvement of tropical forest trees.

New means to overcome the physical difficulties of studying the flowering and genetics of large trees were reported. P. S. Ashton (University of Aberdeen) described a system of spars and rigging and bosun's chairs used in a joint project of the Universities of Aberdeen and Malaya to study pollination in the canopy of a rainforest. Other speakers reported less gymnastic methods in forest genetics. A. N. Start and A. G. Marshall, of the University of Aberdeen, examined pollen in excreta of bats at their roosts to see which trees species they visited and how far they carried pollen. J. Burley, of Oxford University, used extensive progeny tests and analysed isozymes from endosperm and terpenes from resin in seeking patterns of genetic variation in tropical pines.

The IUFRO workshop in Nigeria and Ghana "Variation and Breeding Systems of *Triplochiton scleroxylon*" presented the early results of concentrated research for the domestication of one tree. This fast-growing species is common in secondary forests in the humid tropics of West Africa. Although it is the major export timber of Nigeria it has been difficult to re-establish after cutting.

Recent field studies conducted by N. Jones, of the Federal Department of Forest Research, Ibadan, have shown that irregular production of seed is largely due to variation in the abundance of a weevil and a smut fungus which destroy the developing seeds. Control of these parasites by collecting the fruit when it is still green can provide enough seed for a regular plantation programme.

N. Howland, also of FDFR, Ibadan, reported the development of a second means of establishing *Triplochiton* plantations by mass production of rooted cuttings from seedlings.

The meeting at Oxford and in West Africa brought out the need for a series of representative reserves to try to maintain a large part of the genetic variation in each of several tropical species. In heavily populated areas it is hard to keep reserves of natural forest which seem to be unused. Farmers demand to clear them for food crops. Even foresters are increasingly obliged to clear them for plantations of exotic trees. The contention that fast-growing plantations will take the pressure off the natural forests was disputed by L. Roche, of the University of Ibadan, and other speakers who gave examples of plantations replacing natural forests, not saving them.

What is to be done? The obvious answer is a strong and comprehensive policy of land use, a political not a biological solution. The scientist's special contribution is to provide governments with biological evidence to support rational political and administrative action.

Fe-S clusters in chemistry and biology

from D. O. Hall

THE chemistry of the Fe-S clusters which form the active centres of ferredoxins is a blossoming field. Impetus has come in particular from Holm's group at the Massachusetts Institute of Technology who have synthesised analogues of the two types of cluster which are found in ferredoxins and have recently been able to wrap nine- and twelve-chain peptides around the synthetic compounds (*Endeavour*, **34**, 38; 1975). This and other work is shedding new light on the properties of Fe-S centres.

The iron-sulphur proteins, of which the ferredoxins are an important group, are found in all species and catalyse a wide variety of metabolic reactions. They have the important ability to transfer electrons at very low potentials: between -600 and -400 mV. They can form redox chains with haem and flavin proteins and can also form complexes with flavin and molybdenum in a single molecule.

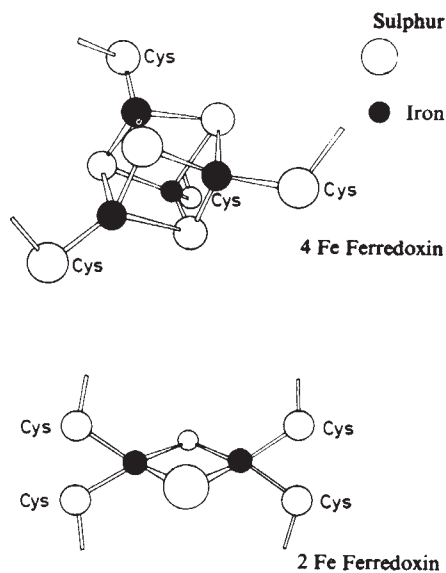
These proteins contain equivalent amounts of sulphur and non-haem iron (attached to cysteine) in their active centres. The two types of cluster found in ferredoxins contain respectively 4Fe-4S and 2Fe-2S (see figure). Until about five years ago their lability made them difficult to study; the situation changed with the advent of techniques such as electron paramagnetic resonance and Mössbauer spectroscopy.

X-ray crystallography of proteins containing two 4Fe-4S-type clusters (one a ferredoxin containing 8Fe in

two clusters from a clostridial-type bacterium; the other a high-potential iron protein (HiPIP) from *Chromatium*) revealed similar structures. Yet the first had a redox potential of -380 mV and the second a redox potential of $+350$ mV. To explain such similarity in structure and difference in redox potential between the ferredoxin and the HiPIP, Carter (University of California, San Diego) proposed that each cluster could exist in three redox states, with C^-/C at -400 mV and C/C^+ at $+350$ mV.

Cammack (*Biochem. Biophys. Res. Commun.*, **54**, 548; 1973) provided evidence for this hypothesis by treating reduced HiPIP with dimethylsulphoxide (which denatures the polypeptide chain but not the Fe-S cluster) and finding that the cluster was reversibly reduced from the C to the C^- form. Sweeney *et al.* (*Biochem. Biophys. Res. Commun.*, **59**, 188; 1974) have found 8Fe ferredoxin in the C^+ state predicted by the hypothesis to exist, but have not demonstrated that the change to the C^+ form is reversible. Studies of the analogue Fe-S compounds (Herskovitz *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **69**, 2437; 1972) and Mössbauer spectroscopy of an 8Fe ferredoxin, a 4Fe ferredoxin and HiPIP (Johnson *et al.*, *Biochem. J.*, **139**, 97 and 105; 1974; and 1975, in the press) suggested that the formal valencies of the iron in the three states are: C^- , $3Fe^{2+}$ and $1Fe^{3+}$; C , $2Fe^{2+}$ and $2Fe^{3+}$; C^+ , $1Fe^{2+}$ and $3Fe^{3+}$. But strong covalent bonding between the H atoms means that the atoms within a cluster behave nearly equivalently.

Returning to the analogue compounds, where the developments have been striking: Holm's group has been able to synthesise the analogue of the 4Fe-4S cluster that occurs in ferredoxins. It is a dianion tetranuclear alkyl thiolate-liganded 4Fe-4S cluster, which has electronic, magnetic and structural properties very similar to the naturally occurring 4Fe-4S cluster found in ferredoxins. Wrapping a peptide around the cluster was accomplished by ligand substitution for those ligands that originally formed the synthetic analogue. In designing the peptide to wrap around the synthetic analogues they made use of the knowledge that the cysteines to which the iron atoms bind are always separated by two (or three) other amino acids giving $-cys-x-x-cys$. Holm *et al.* used glycine-cysteine peptides to wrap around the cluster. They have also synthesised a 2Fe-2S analogue which is a dianion. The Fe-Fe distances in the 4Fe-4S analogue are 2.75 Å and in the 2Fe-2S analogues are 2.70 Å. Since we do not as yet have the X-ray structure of a 2Fe ferredoxin, this 2Fe-2S synthetic analogue is the first actual struc-



ture of this type of centre—even though we do know from theoretical and physical-chemical studies of this 2Fe ferredoxin that the iron atoms interact antiferromagnetically and that since each iron is distinctive only one of them is able to add and subtract electrons (Gibson *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **56**, 987; 1966).

A further recent development has been work by Que, Holm and Mortenson (*J. Am. Chem. Soc.*, **97**, 463; 1975) who have by ligand substitution extracted the Fe-S clusters from 8Fe and from 2Fe ferredoxins. In the former case they obtained the 4Fe-4S core and in the latter case, of course, the 2Fe-2S core. Using the correct ligands they are able to maintain these cores in isolation and characterise them spectrophotometrically. Interestingly the isolated 2Fe-2S core can be converted to the 4Fe-4S core under certain conditions. Complementary work has been done by Bale and Orme-Johnson (Madison, Wisconsin) with similar ferredoxins.

Bruice *et al.* (*Proc. natn. Acad. Sci. U.S.A.*, **72**, 231; 1975) have looked at the acid-base properties of the 4Fe-4S clusters and have come to the interesting conclusion that the tetranuclear Fe-S clusters exist in solution as acid-base pairs with a pK_a of about 3.9, comparable to formic acid. Thus at a given pH of the medium the protein, by influencing the pK_a , could control oxidation-reduction reactions catalysed by these Fe-S centres, and they have shown that there must be at least two proton associations that go on during the oxidation-reduction of 4Fe-4S clusters. They have proposed a mechanism whereby the dianion takes on a proton to form a conjugate acid which then opens spontaneously by a reversible fission of two of the Fe-S bonds;

this compound in turn reversibly associates with another proton to provide its conjugate acid, which spontaneously unfolds through the fission of another two Fe-S bonds yielding a different species. They suggest that the proton associates with one of the six faces of the dianion cluster which occurs through a hydrogen bridge structure above the face of the cluster; they propose that the proton hops or tunnels from face to face since each of the six faces is identical around the periphery of the Fe-S cluster. The practical implications of this as far as the proton is concerned are that the acid species is more susceptible to oxidation by oxygen, and in the Fe-S proteins one should consider that the protein itself controls the pK of the cluster, which in turn controls the ratio of the oxidised and reduced states at any given pH. They propose that the reduced state is a relatively strong acid and conceive that it could have a role as a general acid in enzyme catalysis. These ideas are very interesting in view of the widespread occurrence of Fe-S centres in both soluble and membrane-bound states involved in electron transport coupled to oxidative and photosynthetic phosphorylation. It is possible that similar roles may also be found for Fe-S centres in other types of metabolic reactions.

Now that organic chemists can synthesise analogues of Fe-S centres and wrap a peptide chain around them, and now that we know that these clusters can be removed intact from the protein and also alter their redox potentials while they are still in the peptide, there is scope for wild speculation that we are really on the road towards a synthetic biological catalyst. Outside the realm of biology it is just conceivable that Fe-S clusters could substitute for catalysts like platinum and other compounds which operate at the potential of the hydrogen electrode. After all, Fe-S centres have been functioning adaptively for the last $3\frac{1}{2}$ thousand million years; perhaps it is time they were put to new uses even more diverse from their biological ones than their biological ones are from each other.

Ambiguities in helion optical potentials

from P. E. Hodgson

DURING the last few years there has been much interest in the determination of the best optical potentials to describe the elastic scattering of helions and alpha-particles by nuclei. It is found that several potentials of different depths will fit equally well the dif-

ferential cross section for a range of forward angles but that, if data are available beyond a certain critical angle depending on the target nucleus and the incident energy, then only one potential will fit these data, and the ambiguity is thereby resolved (*Nature*, **239**, 66; 1972: **240**, 1; 1972: **244**, 390; 1972). In this way it has been shown in a series of analyses that the potential with the volume integral per nucleon of the real part about 330 MeV fm^3 is to be preferred over that having a volume integral of 440 MeV fm^3 .

At the same time as this work on potential ambiguities there have been many studies of two-step contributions to nuclear reactions (*Nature*, **247**, 179; 1974: **251**, 188; 1974). It is impossible to understand many nuclear reactions by supposing that they can go only by a direct process from the initial to the final state. In addition, there are contributions from two-step processes that go by way of an intermediate state of the target nucleus, the residual nucleus, or of another nucleus formed from the target by a nucleon transfer process. If these are included in the calculation a good fit into the data can be obtained.

These two lines of work have recently been brought together by Shepard, Kunz and Kraushaar of the University of Colorado (*Phys. Lett.*, **56B**, 135; 1975). They have pointed out that two-step processes probably contribute to elastic scattering as well as to reactions, and go on to study what effect this has on the optimum optical potential.

They consider the elastic scattering of 83.5-MeV helions by ^{58}Ni , and make an optical model analysis of the differential cross section, which is available from 8° to 113° . This range includes sufficient high-angle data to resolve the ambiguities between the different potentials that all fit the forward angle data and they found that a satisfactory fit is obtained only with the shallow potential with volume integral 330 MeV fm^3 . The deeper potential does not fit the data at all well at the higher angles. The result is in line with many previous analyses preferring the shallower of the two potentials.

Further calculations were then made with the inclusion of coupling to the alpha-particle channel by the process (h, α) (α, h) in which the incident helion picks up a neutron and then gives it back to the target nucleus before being emitted into the elastic channel. This two-step process can go by way of several states in ^{57}Ni , and the total amplitude was calculated using the coupled-channels formalism taking into account three intermediate states of ^{57}Ni .

These two-step amplitudes were then added to the direct optical model amplitudes, and the analysis of the elastic

scattering data repeated. An acceptable fit was now obtained with the deep potential, contrary to the previous result.

This result shows that the explicit inclusion of two-step processes in the analysis of elastic helion scattering has a marked effect on the optical potential, even altering the choice of physical potential. This situation forces us to consider carefully what we mean by the optical potential (*Nature*, **249**, 412; 1974). Is it the potential that gives the best fit to a particular cross section or to a range of cross sections for many nuclei, or is it the potential that best fits the data when allowance is made for particular features of the nuclei concerned or after certain second-order processes have been removed?

It is quite arbitrary to say that one of these potentials is more physical than the others, but it is important to remember the conditions under which it was obtained and then see how well it fits other data, particularly the cross sections of nucleon transfer reactions. The analyses of such data give different results depending on the inclusion of two-step and other higher order processes. It is indeed already found that the deeper potentials give a better description of some two-nucleon transfer reactions than the shallower ones, indicating that it might be preferable to include two-step processes explicitly when obtaining optical model potentials by analysing differential elastic scattering cross sections. The important thing is to ensure overall consistency, avoiding the danger of implicitly taking account of some processes more than once, and as far as possible fitting both elastic scattering and reaction data with the same potentials. There is clearly much more work to be done along these lines to find the best way of analysing the data.

Fertiliser for red grouse

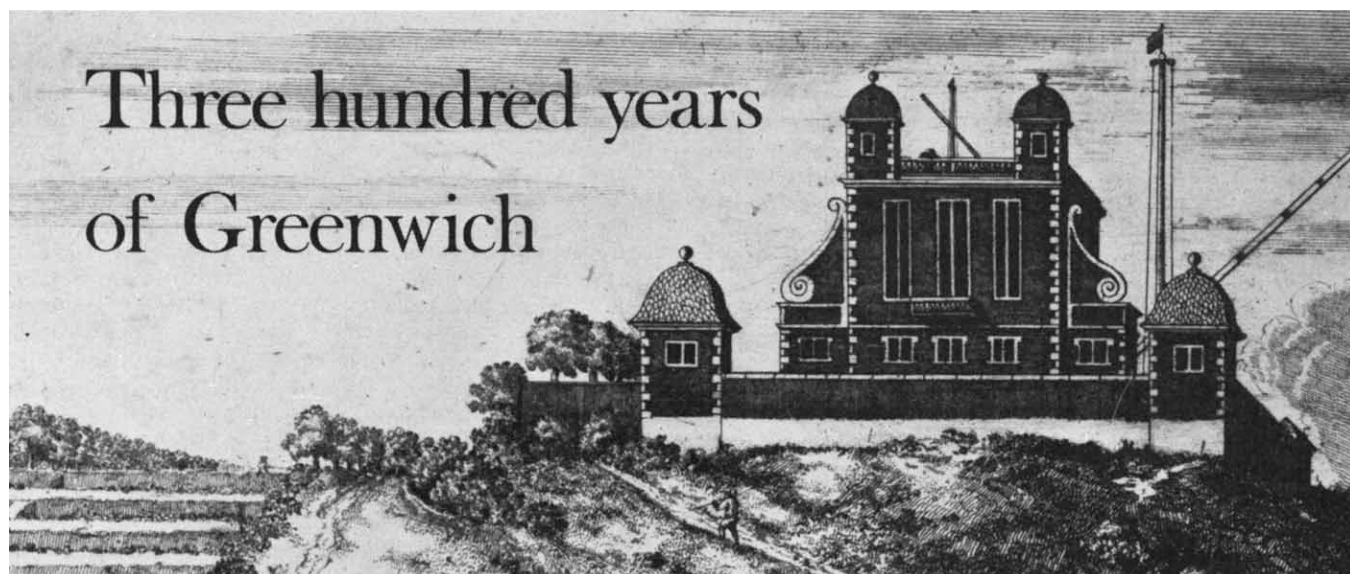
from our Animal Ecology Correspondent

THE level of secondary productivity in an ecosystem is clearly dependent upon the level of primary productivity. This axiom has been tested in aquatic ecosystems where the application of fertilisers resulted in a higher yield of fish (Mann, *J. Anim. Ecol.*, **34**, 253; 1965). Few such studies have been conducted on terrestrial vertebrates, but one of the most thorough is the long-term project in northern Scotland on the red grouse (*Lagopus l. scoticus*). Grouse inhabit open moorland where the dominant plant species is heather, *Calluna vulgaris*. Heather is a fortunate species for nutritional studies since it shows a clearly defined annual growth pattern.

Miller, Watson and Jenkins (*Animal Populations in Relation to their Food Resources*, edit. by Watson, 323; Blackwell, 1970) showed that the overall growth of the heather influenced the breeding success of the birds, that is, the number of surviving young per pair of breeding adults. The traditional practice of burning the heather to encourage further growth resulted in a higher density of birds on the moor but had no effect on breeding success: the increased density was probably the result of enhanced survival of resident birds. An experimental plot of 16 Ha was treated with 105 Kg ammonium nitrate per Ha. This had two effects on the production of heather which have greatly helped our understanding of the fertiliser's influence on secondary productivity. The first was that the N content of the growing tips was significantly increased. The second was that growth started earlier in the spring. On the fertilised plot there was a markedly higher breeding success than on an adjacent control plot, which brought about a three-fold increase in population density.

In a recent analysis of grouse breeding success, Moss and his colleagues have examined the relationship of maternal nutrition (primarily the N and P composition of heather), the date when the heather started to grow and the quantity of heather available with the breeding success on the moor (*J. Anim. Ecol.*, **44**, 233; 1975). Data from six grouse moors gathered over six years showed that there was a just significant correlation between mean nitrogen content of heather and mean breeding success but no significant correlation with respect to phosphorous was noted. There was further no significant correlation between breeding success and N and P levels when the data were analysed on a year-to-year basis. These nutritional factors were strongly correlated with the number of breeding birds, however, probably as a result of their effect on survival. What the data did show was that the density of heather available to the breeding grouse and the length of time it has to grow before the start of breeding was strongly correlated with breeding success from year to year.

The implications of this analysis are interesting. It would seem that the addition of ammonium nitrate fertiliser to grouse moors results in increased breeding success and population density through a slight change in the chemical composition of the plants and a strong influence on the date of commencement of spring growth, and, hence, the quantity available. It will be interesting to see if those undertaking other studies, particularly with aquatic ecosystems, come up with similar conclusions.



Graham Smith : The next 300 years • John Russell : English astronomy before 1675
Eric Forbes : The first 150 years • Jack Meadows : Airy and after
Alan Hunter : The twentieth century • Philip Laurie : Some Greenwich sidelights
Owen Gingerich : The astronomical scene in 1675

THREE hundred glorious years have gone by, full of purpose and achievement. Inevitably one is asked, and one asks oneself, what of the next 300 years? Astronomers spend an appreciable proportion of their lives denying all connection with astrology, but the prediction of the distant future needs a crystal ball rather than a rational approach. One can only try the well known and dangerous trick of extrapolation, treading on ground which becomes more nebulous and dangerous the further one looks ahead.

Extrapolations need as many terms in a series as one dare assign coefficients. The zero order term in this case—the present state of British astronomy in general and the Royal Greenwich Observatory (RGO) in particular—is available, and corresponds to a good state of health. The first differential is clear when one looks at the changing roles of the universities and the Royal Observatories, at the growth of opportunities for observations using large modern telescopes, and at the present excitement in astrophysics. The second differential is, however, problematical. A look back into history will show that such changes of direction have occurred on so many occasions in the last century that extrapolation would have been impossible for more than a decade or two. What are the factors which stimulate these changes?

The growth of the observatory in the early eighteenth century was a response to the very clear need for accurate navigational methods. Within this general background there was a varying combination of direct help to mariners and research into improved techniques. The results have been summed up in the Stationery Office publication *Man is Not Lost*, by D. H. Sadler. The famous history of determination of the Moon's motion, and the efforts to obtain unprecedented accuracy in stellar positions through the succession of wonderfully accurate, meridian transit instruments at Greenwich, must have been, in their day, as absorbing and as rewarding as we today find the pursuit of astrophysical conditions within the stars, or the delineation of

the geometry of the Universe. But the justification for these labours was well known from the start, and if the Astronomers Royal of those days ever had to take any time from their labours to justify their expenditures to the Board of Ordnance or the Admiralty, one can only envy them the simplicity of their task. The life of a Director of the Observatory is surely very much more complicated in these days, partly because of the diversity of the work and the number of people within his care, but partly also because the nature of scientific work and its relation to modern society is so different.

The complications in the research programme of the observatory date back to Christie, who introduced an astrophysical element to the previous concentration on positional astronomy and time. The extent of this discontinuity only became obvious when the Isaac Newton Telescope came to be regarded as the most important asset of the observatory: even then, the telescope itself remained the property of the Science Research Council, and only under the management of the observatory. But the change had been rapid, and for some of the old school it had been hard to swallow. Woolley cut to the bone the manpower and resources devoted to meridian astronomy and to the time service, so much so that these essential functions could only continue with almost no development and rejuvenation. He expanded instead the astrophysical research, encouraged his team to observe with whatever large telescopes they could gain access to, and started the move towards overseas astrophysical observatories which now begins to bear fruit in the Anglo-Australian Telescope.

The point of retelling this history is a reminder that change has been rapid, and that it continues to be rapid. What were the conditions which provided the catalyst and the resources for the change?

Astrophysics grew explosively in the 1950s and 1960s during a surge of optimism and enthusiasm for science and education which was demonstrated best by the expansion

of the universities. There was a time when consternation within the Science Research Council (SRC) committees meant not that the research funds were being cut back, but that the rate of growth of research funds was being reduced. It hardly needs saying that things are different today. Mercifully there are no large cuts in funds for astronomy as a whole, but the days are gone when almost any good research idea could find nourishment from university or SRC establishment funds. The exponential growth had to stop, and the time scale of stopping was shorter than the growth time constant we had become used to.

The role of the RGO in British astronomy has recently been redefined by the SRC. Its prime task is now to provide astronomers throughout Britain with the opportunities and equipment for observational astronomy. It has, of course, a continuing responsibility for the time service, and it has international responsibilities in determining the positions of stars. But if one takes a hard look at the rationale behind the continued existence of the two Royal observatories one must admit that the emphasis has swung heavily over towards the new support function, without which university astronomical departments would scarcely be able to survive more than a few years.

The Northern Hemisphere Observatory

The future of the RGO over the next decade will be dominated by the decision of the SRC that the observatory shall be responsible for the provision of a new overseas observatory, devoted primarily to astrophysical research and organised primarily for the benefit of the universities. This observatory will contain three main telescopes. A very large telescope (4.5 m in diameter) will be dedicated to observations of the faintest extragalactic objects, using the best modern techniques of photon detection. A medium telescope (2.5 m in diameter) will provide a more general workhorse. This will, in fact, be the Isaac Newton Telescope, moved to the new site and improved by a new primary mirror which increases its effective diameter from 96 to 100 inches. The third telescope (1 m in diameter) will be used for photometry and astrometry.

The new observatory will be placed on a first class site at a low northern latitude. It should be possible to triple the useable hours as compared with Herstmonceux. Furthermore, the clarity of the sky and dramatically reduced

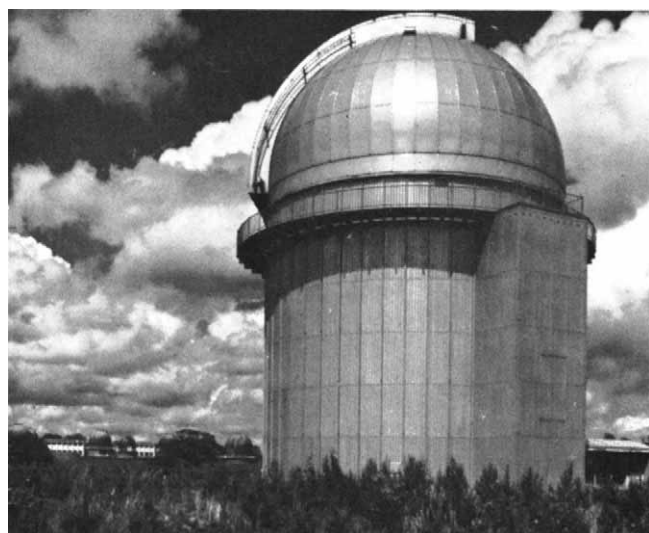
scintillation will improve the sensitivity of the Isaac Newton Telescope by an order of magnitude; it will be at least among the best in the whole world for observing faint objects. But which site will be good enough, and available? Three possible sites are being examined: Mauna Kea in Hawaii, La Palma in the Canary Islands, and Madeira. All three are volcanic peaks on oceanic islands, at altitudes well above the wet, polluted atmosphere which is confined below an inversion layer. Hawaii is fast becoming a centre for astronomy: a British infrared telescope is being installed there, under the guidance of the Royal Observatory Edinburgh, where it will join a French-Canadian-Hawaiian 4-m optical telescope. La Palma may well become a European centre: the Spanish Research Council is considering the possibility of inviting several countries, including Britain, to install telescopes in a new observatory on the mountain top. A joint European site-testing team is producing very favourable reports on the suitability of this location, which at latitude 28.5°N is well within the belt of temperate latitudes which have a particularly favourable climate. Evidently, Hawaii and the Canary Islands both offer excellent sites; Madeira is not quite as good, but it is not far behind in quality. There is a good chance that a choice of a first class site can be made by the end of 1975.

I therefore see a rosy future for British astronomy over the next decade or two, with the Anglo-Australian Telescope and the Northern Hemisphere Observatory providing observing opportunities which have been long denied to us. I also see the two Royal observatories as playing a vital role in the provision and maintenance of the new overseas telescopes. Furthermore, it is essential that these telescopes should be specified, built, operated, maintained and improved under the influence and stimulus of observational astrophysicists, who have an interest in using the telescopes rather than regarding them as mere status symbols. It is agreed, therefore, that the Royal Observatories will continue to research in astronomy, particularly in subjects which are favoured by the new telescopes, providing resident astronomers at the overseas sites, who will work with the university visitors, and developing new techniques of spectrometry and photon detection as the needs of modern observations become apparent.

Now let us turn to the crystal ball, and look for the next change of direction, which may be taking us into the next century. What will be the social pattern in Britain, which more than any aspirations of the scientific community will determine the pattern of the RGO's work? The days when commerce, exploration and the navy all combined to demand a new navigational service are gone. So are the days when the RGO could be left to its own devices, developing its own research as an independent institution. Today, it is dependent on the universities. The money comes, of course, from the SRC but that is largely for the benefit of British science in general and the universities in particular. Surely, one must assume that this is a healthy position, and that it will continue for the foreseeable future: if we believe in science and education, and in the university system, then we must provide essential facilities for research.

If it is accepted that modern conditions are reasonably well met by the present organisation of British astronomy, it must be asked whether the right kind of astronomy is being done, and whether there will be changes in techniques which will, or ought to be, taken up by the Royal Observatories. What, in particular, about space astronomy? Here there will undoubtedly be changes. The first Large Space Telescope (LST) is well past the initial design stages, and even though it is not yet funded, and even though its specification has shrunk from a diameter of 3 metres to a diameter of 2.3 metres, with less than perfect angular resolution, there can be no doubt that some such telescope will be in orbit not long after the new Northern Hemisphere

The 98-inch Isaac Newton telescope at Herstmonceux
[Photo: David Calvert].



Observatory comes into full operation. Will that represent an eclipse? Must Britain choose between the enormous expense of space work and closing up observational astronomy after a few glorious years of front line, ground based work?

A previous Director of the RGO, in an incautious moment, said "Space travel is utter bilge". He was partly right. Where today are the streams of astronauts colonising the planets, and exploring the newly discovered civilisations on the nearest stars? How many men are even in orbit round the Earth at this moment? Space telescopes, however, are certainly not bilge, and we certainly expect and hope to see several of them in orbit at the end of the century. They do, however, assume a seductive character which is used as an excuse for delaying vital ground based telescope projects. In no sense are space telescopes going to replace ground based telescopes, like a kind of celestial beam trawler which sucks up all the good fish in front of a few hapless, poverty stricken, local fishermen. There is no such thing as a universal telescope which will sweep all the good things out of the sky.

The LST will have particular capabilities in ultraviolet astronomy because of its location above the absorbing atmosphere, and it will have the possibility of unprecedented angular resolution because of the lack of atmospheric scintillation. But many observations require neither of these advantages. For example, the spectrum of a galactic nucleus is interesting mainly in the optical band, and the nucleus is often larger than the 'seeing disk' because of scintillation. So that particular job, and others like it, could not reasonably be assigned to a telescope which has an initial cost that is more than 10 times the cost of the whole new Northern Hemisphere Observatory, and which will have running costs and a lifetime that are scarcely predictable. It would be better done on the larger, more convenient, and specially designed 4.5-m telescope. Examples could be multiplied *ad nauseam*; the present assessment is that three-quarters of astronomy can be done at least as well from the ground as from satellites. Obviously, we will choose the ground based techniques, which are cheaper, more direct, more adaptable, and, most importantly, most easily available to a large body of university observers. Postgraduate students already find it hard to get a look in at the major ground based telescopes; shall we seriously consider financing them to work only in the large teams which surround the billion dollar space projects? The answer is obvious. Practically no new technique which has been introduced into astronomy over the post-war years has chased out previous techniques. Radio and X-ray astronomers are clamouring for observations on large optical telescopes, and even for improved

astrometric work on the old fashioned pattern. The NASA report of 1969, *A Long-range Program in Space Astronomy*, was quite specific on this point:

'First of all, the existing ground based observing centres and university departments are the principal source of manpower and the training ground for young astronomers needed in astronomical projects involving observations from space . . .

'The space program depends on ground based astronomy in a second way: selecting the crucial observations that will yield the most useful return from the specialised instruments sent above the atmosphere draws on a vast body of data accumulated from studies of many types of objects by optical and radio telescopes. This reservoir of information is continually being renewed and augmented . . .

'Finally, ground based telescopes offer valuable direct support and supplementation to orbiting space instruments.'

The report concludes by recommending the construction of two new observatories, in each of which there should be a 200-inch (5-m) telescope.

Prophets face a dilemma shared by the weather forecasters. Either they make themselves look ridiculous, by chancing their arms and risking being proved wrong, or they predict, more safely, that tomorrow's weather will be much the same as today's in which case they are at least more likely to be right than wrong. In either case, they must ignore the dire warnings of imminent catastrophe in the form of a sudden ice age. I have taken the cautious view that astronomy is such a basic science that no society will fail to support it: I have assumed that universities will continue to do research: I have assumed that astronomy provides the best general education and inspiration in science at school level. If I am right, and if no catastrophic ice age descends upon us, the future of astronomy and of the Royal Greenwich Observatory in particular is as bright as it has ever been during the past 300 years.

Graham Smith

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English astronomy before 1675

John L. Russell

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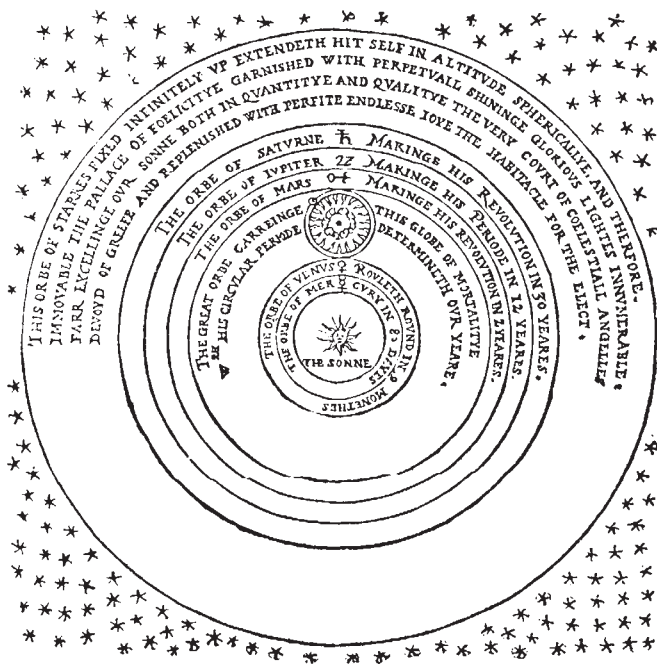
Although the RGO has been at the centre of British astronomy for 300 years, there was astronomy in Britain before 1675.

THE history of English astronomy before 1675 has certain features which distinguish it from other European countries. In contrast to, say, Italy, Germany and the Low Countries, the study of science and mathematics, at least until the mid-seventeenth century, was largely independent of the

universities. Our knowledge of the day to day teaching in Oxford and Cambridge during the sixteenth and seventeenth centuries is still rather incomplete but it does not seem that undergraduates at either university received any regular instruction in astronomy, or any but the most elementary mathematics, during most of this period. After 1619, with the founding of the Savilian Professorships, postgraduate students at Oxford working for the degree of MA were provided with good courses in geometry and mathematical astronomy but the teaching in other subjects

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Fig. 1 Thomas Digges's diagram of the infinite Copernican Universe.

remained very traditional. There seems to have been comparatively little stimulus to research or openness to new ideas until the late 1640s. At Cambridge the mathematical and physical sciences were even more neglected until the appointment of Isaac Barrow as the first Lucasian Professor of Mathematics in 1663. Some students were fortunate enough to find college tutors who could help them in these subjects but, on the whole, opportunities were restricted.

First astronomy courses

Regular courses in astronomy and geometry were, however, available in London from 1597 at Gresham College, which numbered several distinguished astronomers and mathematicians among its professors during the seventeenth century. The courses were mainly practical in intention, being designed primarily to train mariners, cartographers, instrument makers and others for whom a knowledge of astronomy would be of practical value. In addition, however, the college provided a centre where other scientists, not necessarily students of the college, could meet together for discussion and, later, for research.

Neither the universities nor Gresham College made any important original contributions to astronomy before the middle of the seventeenth century. But there was some significant research carried on elsewhere, often by smaller groups of scholars working together. One such group was led by John Dee (1527–1608) from about 1560 onwards. His house at Mortlake contained a first class scientific library, freely available to enquirers. He was a mathematician with a European reputation and many international contacts, so that he was able to keep his circle abreast of developments elsewhere: a valuable asset at a time when England was somewhat isolated intellectually from the Continent. He recognised the inadequacy of the

mediaeval astronomical tables and when, in 1551, the German astronomer Erasmus Reinhold published his improved "Prussian Tables" Dee was quick to see their value and to encourage his friend John Feild to compute a small table of ephemerides based on them. These were published in 1556.

Another member of Dee's circle was Thomas Digges (c. 1546–95), the most important English astronomer of the sixteenth century. In his *Alae seu scalae mathematicae* (London, 1573) he described the new star of 1572 and measured its position with an accuracy which won praise from Tycho Brahe, the acknowledged expert in this field. His measurements showed that the star had no detectable diurnal parallax and must therefore be beyond the moon, in the celestial regions. Three years later, Digges also made a valuable contribution to astronomical education by translating into English a part of Copernicus's *De revolutionibus* (1543) in which the basic features of the heliocentric system were clearly expounded and defended. This was entitled *A perfit description of the caelestiall orbes* (London, 1576) and was accompanied by a diagram of the system (Fig. 1) which has a special interest since it contains the earliest assertion, within the context of the new astronomy, that the Universe is infinite in extent.

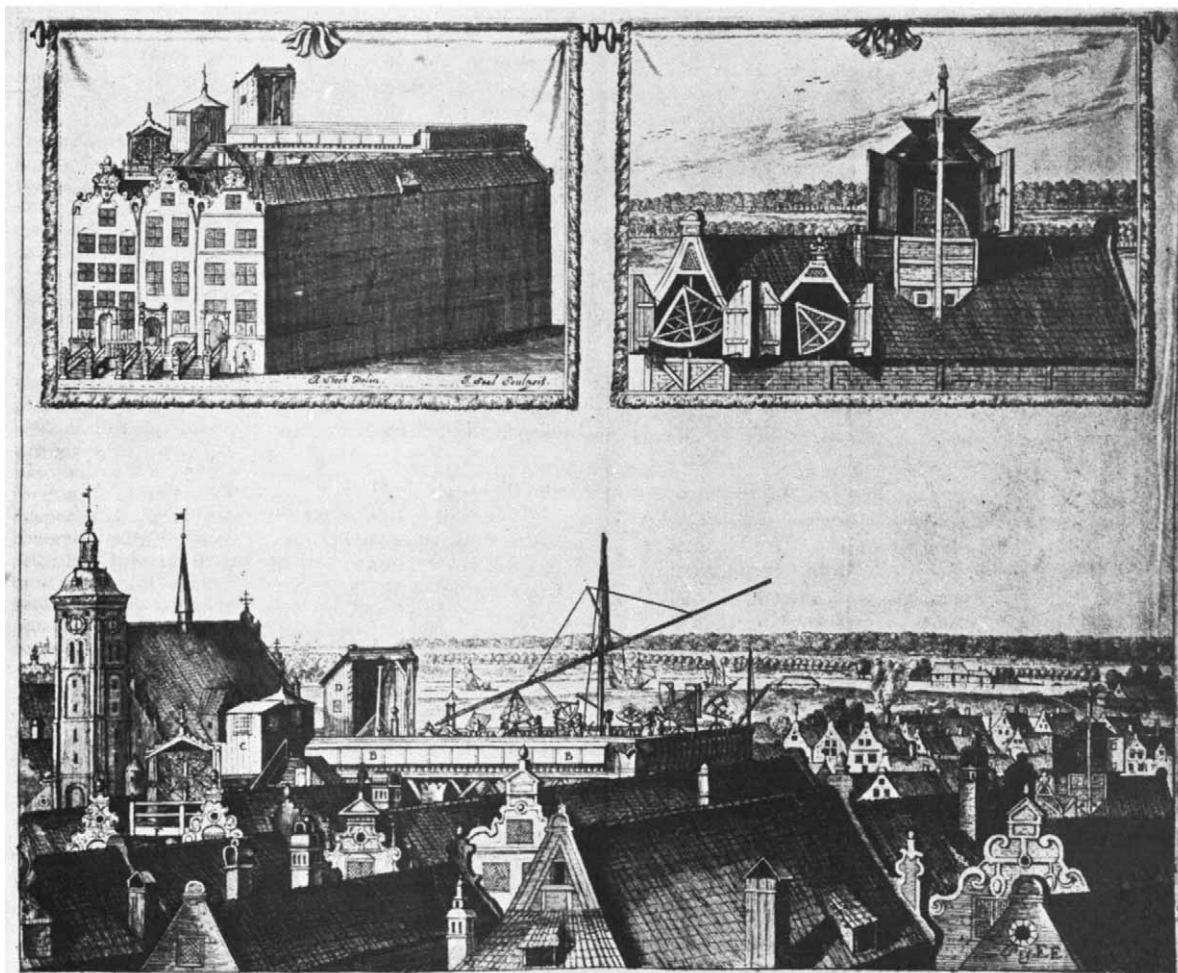
Observational studies

One other more practical contribution to the science of astronomy was made in England during the sixteenth century. This was Edward Wright's careful determination of solar declinations throughout the year, based on a series of observations extending over the years 1594–97 and published in his *Certain errors in navigation* . . . (London, 1599). They were undertaken primarily in order to give navigators more accurate data for determining latitudes at sea. He used a large quadrant with a radius of more than 6 feet, graduated in minutes and half minutes. Its accuracy can be gauged from his determination of the latitude of London for which he obtained a value of $51^{\circ} 32'$, which is within $2'$ of the correct figure and much better than the previously accepted value.

Wright's quadrant seems to have had a further history. More than 20 years later, between 1617 and 1621, Andrew Young was making equally accurate meridian observations at Edinburgh, including a measurement of the latitude of that city which was correct to about $1'$. There are good, though not conclusive reasons for thinking that Young was using Wright's quadrant. It is known that some time after Wright's death in 1615 his friend, Henry Briggs, sent a large quadrant from London to John Napier at Merchiston, near Edinburgh, and that it was later set up in the Old College at Edinburgh. It is not certain either that this was Wright's quadrant or that it was the one used by Young but both suppositions seem highly probable. Its subsequent history is unknown.

Napier's invention of logarithms in 1614 and Briggs's improved versions of Napier's tables in 1617 and 1624 should also be regarded as major indirect contributions to astronomy since they greatly facilitated the complicated trigonometrical calculations required for the compilation of planetary tables. Kepler, in particular, made extensive use of them in preparing his famous Rudolphine Tables.

The only other astronomical observations of any moment published by an English astronomer before the 1660s were contained in a small work by John Bainbridge: *An astronomical description of the late comet from the 18. of Nouemb. 1618. to the 16. of December following* (London, 1619). He plotted its path between these two dates by means of a cross-staff and made more detailed observations through a telescope on December 3 when it was close to two stars in the constellation of Bootes. Using these as a comparison, he was able to establish that the diurnal parallax of the comet was undetectable—certainly less than $6'$ —which



THIS illustration shows the telescope and instruments of Johannes Hevelius (1611–1687) rising above the rooftops of Danzig; it is taken from his *Machina coelestis* of 1673 (by permission of Harvard College Library). Hevelius was a wealthy man and established

the best equipped observatory in Europe in 1641. The observatory included his famous tubeless telescope (which had a focal length of 150 feet) but was destroyed by fire in 1679; undaunted Hevelius built and equipped another.

placed it well beyond the Moon in the celestial regions. He also described the appearance of the comet in some detail.

Theoretical work

The most important contribution to theoretical astronomy published in Britain before 1650 was contained in William Gilbert's great work *De magnete* (London, 1600). This was chiefly concerned with the phenomena of experimental and terrestrial magnetism but he concluded his treatise with some speculations on its possible cosmological significance, suggesting, in particular, that the planets are kept in their courses by magnetic forces. He made no attempt to work out this idea in detail and its main importance lies in the fact that it stimulated Kepler to devise his own 'quasi-magnetic' theory of planetary motions. Although this theory was incorrect it played a major role in guiding him to the discovery of his first two laws of planetary motion. Gilbert also argued vigorously and cogently in favour of a diurnal rotation of the Earth, and his arguments undoubtedly converted many of his fellow scientists to this view. On the question of an annual orbital motion round the Sun he never committed himself one way or the other. Partly on this account, many scientists in the early seventeenth century accepted a 'semi-Copernican' view in which the Earth remained at the centre of the Universe but rotated on its own axis. But by 1640 English astronomers were coming

round more and more to the full Copernican theory which was defended in print by John Wilkins in *A discourse concerning a new planet* (London, 1640); by Henry More in *Psychathanasia* (Cambridge, 1642); and by Thomas White in *De Mundo* (Paris, 1642).

Overall progress

If we are to judge the English contributions to astronomy before 1650 solely by published work, the total sum is relatively modest: some accurate observations on the nova of 1572 and the comet of 1618; Wright's table of solar declinations; Briggs's improved logarithms; Gilbert's speculations on the possible significance of magnetism in planetary theory. These almost complete the list and they can hardly be compared with the achievements of Italy, Germany or France during the same period. There are, however, two other considerations which redress the balance to some extent. The first is that there was probably a more widespread interest in, and a greater receptivity towards the new astronomy, and especially towards the idea of a moving Earth, in Britain than in most other countries. Several writers supported either a full or a semi-Copernican theory; others referred to it sympathetically; few actively opposed it. English support undoubtedly strengthened the cause of Copernicanism elsewhere at this time.

The second balancing factor is that there were, in fact,

two English astronomers of outstanding ability who did first class research in the subject before 1650 but who, for different reasons, failed to publish it. These were Thomas Harriot and Jeremiah Horrox. Thomas Harriot, like his friend Edward Wright, was interested in precise astronomical measurement for navigational purposes but he was also dedicated to astronomical research for its own sake. As early as the summer of 1609, independently of Galileo, he was observing the surface of the Moon through a telescope. During the next four years he made detailed moon maps, observed Jupiter and its satellites over a long period, and made many drawings of sunspots. He was studying Kepler's planetary theory only a few weeks after its publication in 1609 and apparently accepted its mathematical conclusions as substantially sound. Unfortunately he made no attempt to publish any of his scientific research, which has remained in manuscript ever since. Only recently has the task of editing his papers for eventual publication been seriously begun.

Undoubtedly the greatest English astronomer of the pre-1675 period was Jeremiah Horrox who, if he had lived longer and had attracted the notice which he deserved, would have revolutionised the science of astronomy during the 1640s. He was born at Toxteth in Lancashire, probably in 1618, and studied at Cambridge from 1632 to 1635 where, as he tells us, he got no help in his astronomical or mathematical studies. He returned to Toxteth where he lived for the rest of his life apart from a few months in 1639 spent at the nearby village of Hoole, possibly as curate but more probably as tutor to a private family. He died at the age of 22 or 23, in January 1641.

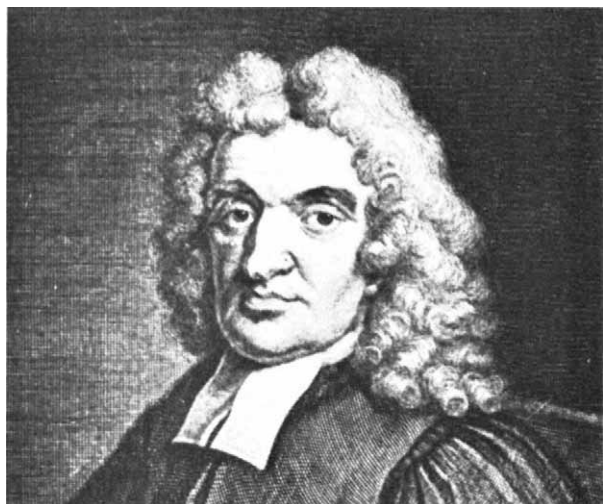
Horrox began his researches in 1635 with the limited purpose of constructing a table of ephemerides based on the tables of the Dutch astronomer Philip Lansberg. He took the precaution of checking some of Lansberg's predictions against his own observations and soon discovered that the tables were of poor quality and that Lansberg's claims as to their merits were grossly exaggerated. In 1636 his attention was drawn to Kepler's Rudolphine Tables which he found to be greatly superior. From then on, he became an enthusiastic disciple of Kepler and, at the time of his death, was engaged on a treatise defending Kepler's theories and urging the general acceptance of his tables. This was published among his posthumous papers in 1672.

Horrox was convinced that Kepler's tables were based on sound theoretical principles but he found that, even so, they were by no means perfect. He realised that they could only

be improved by accumulating more precise data on planetary positions and this he did systematically until his death. As a result, he was the only astronomer to predict a transit of Venus on November 24, 1639 (OS), which had been overlooked by Kepler due to a small systematic error in his tables for that planet. It occurred punctually at the expected time and was carefully observed by him from its beginning until sunset half an hour later. He used the opportunity to make a more precise estimate of the apparent diameter of the planet. By applying Kepler's (incorrect) hypothesis that the absolute diameters of the planets are proportional to their distances from the Sun, he deduced a value for the solar parallax which, though its theoretical basis was unsound, was much closer to the truth than any previous estimate. His most important contribution to astronomy lay in his greatly improved theory of the Moon's motion. Kepler had postulated that the lunar orbit is an ellipse subject to perturbation but he had been unable to find a satisfactory method of treating it as such. Horrox established a basic elliptical path and showed that the main perturbation—the so-called evection—could be accounted for if both the eccentricity and the orientation of the major axis oscillate about their mean values with a period equal to the lunar month.

Horrox worked largely in isolation. His only direct link with others working in the same field was through his friend and fellow Lancastrian William Crabtree (1610–44?) who was also a keen and competent astronomical observer. Crabtree had been the first of the two to discover the merits of Kepler's tables and it was through his advice that Horrox had adopted them. Later, in 1639, he made the acquaintance of yet another young and unknown worker in the same field, William Gascoigne (1612?–44), from Middleton near Leeds. Gascoigne was apparently the first astronomer to realise that in a Keplerian telescope with two convex lenses, as opposed to a Galilean telescope with a convex and a concave lens, it was possible to insert cross wires or measuring instruments in the focal plane where they would give a clear image to the observer. He invented the first micrometer screw gauge for his telescope and made a series of observations on the variations of the apparent diameters of the Sun and Moon. According to his own account, his lenses were not good enough to exploit the full potentialities of his invention but he was able to measure apparent diameters to an accuracy of about 5". He also produced the first telescopic sight by equipping a telescope with cross wires and mounting it on a quadrant or sextant. When Horrox, in 1640, heard of these inventions through Crabtree he was, not unnaturally, filled with enthusiasm but he died before he could make use of them.

Horrox and his friends were exceptional in their clear recognition that the progress of astronomy depended on increased accuracy of observation. Working in almost complete isolation they attained a level of precision which would not be reached elsewhere for another 30 years. If they, and Horrox in particular, had lived longer their influence on the progress of astronomy would have been profound. Unfortunately this was not to be. Horrox died in January 1641 aged about 23, before he had had an opportunity to publish any of his work. Gascoigne was killed in 1644 at the battle of Marston Moor, fighting on the Royalist side. Crabtree probably died in the same year. Many of Horrox's papers were destroyed in the Civil War or were subsequently lost, but some of the most important survived. His account of the transit of Venus was published by Hevelius as an appendix to his own description of the transit of Mercury in 1661: *Mercurius in Sole visus* (Danzig, 1662). Other papers were made available to the Royal Society about 1664. A selection of these was edited by John Wallis and published under the title *Opera postuma* (London, 1672). Gascoigne's micrometer was forgotten until it was independently reinvented in 1666. It was only then that



John Flamsteed, 1646–1719. First Astronomer Royal, 1675–1719 [Courtesy: Harvard College Library].

Richard Townley, who had preserved Gascoigne's original instrument, produced it as proof of his priority.

Prelude to the RGO

For a decade after Horrox's death there was little astronomical research in this country—understandably in view of the disruption caused by the Civil War. About 1650, however, English astronomy entered a new phase. The effort became more sustained, more work was published and there was a greater awareness of the advances which were being made elsewhere in Europe. The immediate stimulus was the rather belated discovery in this country (apart from Horrox and his circle) of Kepler's planetary theory. Kepler had published his first two laws in 1609 and his planetary tables—the *Tabulae Rudolphinae*—in 1627. By 1650 these were generally accepted on the Continent as the best available. The first law, that the planetary orbits are ellipses with the Sun at one focus, was also widely regarded as correct. The situation was different with regard to the second law, which states that the line SP joining the planet to the Sun sweeps out equal areas in equal times. This is a difficult law to apply in practice since it yields no simple exact method of calculating the position of a planet at any given time. The answer must be reached by a rather cumbersome method of approximation. It therefore seemed to be an important advance when the French astronomer Ismael Boulliau, in his *Astronomia philolaica* (Paris, 1645) proposed a simpler and more 'geometric' method of calculating the position of a planet in its orbit.

Kepler's theory and Boulliau's modification of it came to the notice of English astronomers almost simultaneously in the late 1640s and it was Boulliau's rather than Kepler's version of the second law which attracted attention. Between 1650 and 1670 several good planetary tables were published in this country, all of which accepted elliptical orbits. None of them used, or even mentioned Kepler's form of the second law. Instead, various different alternatives were proposed and applied, all of them simpler to use but, in principle, less accurate than the original area law. The first of these tables, Vincent Wing's *Harmonicon coeleste* (London, 1651), substantially followed Boulliau. A few years later Seth Ward, Savilian Professor of Astronomy at Oxford, published his *Astronomia geometrica* (London, 1656) in which he rejected Boulliau's method and proposed an alternative in which the empty focus F of the ellipse was treated as an equant point: a point such that a line FP joining it to the planet rotates with constant angular velocity. This is,

in fact, a good approximation to the area law for ellipses of small eccentricity but diverges appreciably for the more elongated orbits of Mars and Mercury. Boulliau in 1657 accepted some of Ward's criticisms of his earlier work but rightly considered that the latter's proposed variant was insufficiently accurate. He put forward a better alternative in which the equant point was not fixed but oscillated about the focus F . This was accepted by Thomas Streete in his *Astronomia Carolina* (London, 1661). Other modified equant theories were proposed by Nicholas Mercator in *Hypothesis astronomica nova* (London, 1664) and by Vincent Wing in *Astronomia Britannica* (London, 1669).

In a sense one might say that all this effort was directed into a blind alley since Kepler's original area law was, after all, the theoretically correct one. Such a judgment would, however, be misleading. These alternatives were easier to use and did facilitate the construction of new and better planetary tables. The best of them were observationally indistinguishable from the area law within the limits of accuracy available. Horrox himself had used a variant of his own which did not diverge from the true law by more than 10". The main limitation of this type of investigation was that it was purely mathematical. From the time of Tycho Brahe's death in 1601 practically no new accurate determinations of planetary positions had been published. Tycho's best data approached the limits of accuracy which could be attained by naked eye observation but others, especially his earlier ones, were less reliable. Horrox and his circle had been alone in recognising the need for more and better data. By 1670 progress, both in planetary theory and in the construction of tables, absolutely demanded new measurements.

By the late 1660s this was becoming increasingly recognised. Telescopic sights and the micrometer gauge, knowledge of which had died with Gascoigne, were re-invented: the former by Christopher Wren and Robert Hooke about 1665, and the latter by the French astronomer Adrien Auzout in 1666. At the same time Horrox's work was becoming known, even before the publication of his *Opera postuma* in 1672, and was strongly reinforcing the case for greater precision. Flamsteed recognised the importance of his work on the theory of the Moon's motion and prepared new, much improved lunar tables based on it. Planetary theory was entering on a new phase when further advances would require expensive equipment housed in permanent observatories with professional astronomers to staff them. The Royal Observatory at Greenwich was founded as a response to this need.

The Greenwich Observatory: origins and early development, 1675–1835

Eric G. Forbes

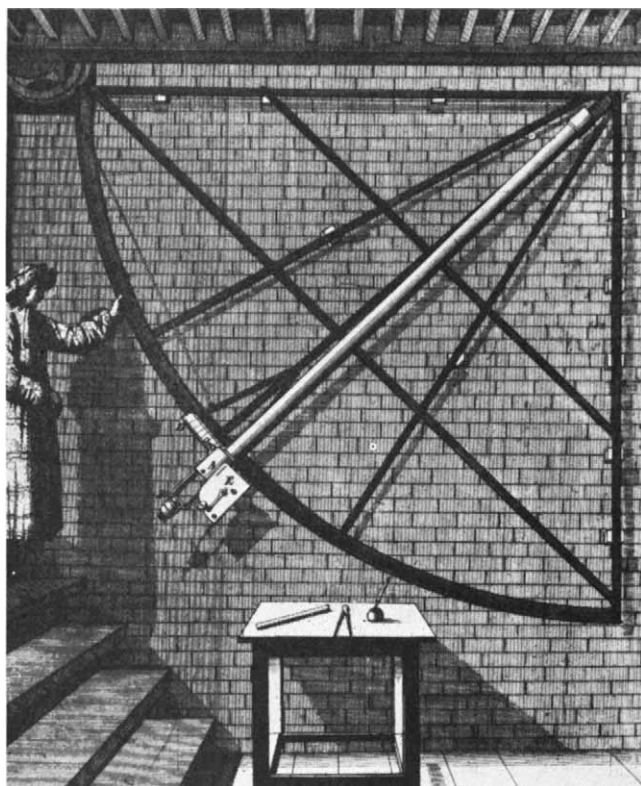
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Eric Forbes considers the influence which the first six Astronomers Royal had on the development of the Royal Greenwich Observatory from its foundation in 1675 until the retirement of John Pond in the nineteenth century.

THE reason for the foundation of the Greenwich Observatory 300 years ago, is clearly and unambiguously defined in the Royal Warrant of Charles II, issued to Sir Thomas Chicheley, Master-General of HM Ordnance on June 22,

1675, as "the finding out of the Longitude of places for perfecting Navigation and Astronomy". To achieve this aim, the King's "Astronomical Observator", John Flamsteed, had "to apply himself with the utmost care & diligence to the rectifying [of] the tables of the Motions of the Heavens and the places of the fixed Stars" for the modest salary of £100 a year.

Why was that observatory established for that purpose at that particular time? The sequence of events which precipitated the foundation of Britain's first institute for scientific research is generally recognised as having stemmed



Hooke's 10-foot mural quadrant, 1676: proved too heavy for use.

from the somewhat rash announcement several months previously by a certain *Sieur de St Pierre*, a Frenchman at the Court of Charles II, that he had discovered a new means of finding longitude at sea from measurements of lunar and stellar altitudes. The King, who had a genuine interest in matters of this nature, was prevailed upon by his favourite mistress, Louise de K  roualle, Duchess of Portsmouth, to issue a warrant on December 15, 1674, to a Royal Society Committee instructing its members, or any four of them, to provide the *Sieur* with precise observations for any specified day, of the altitudes of two stars situated close to the celestial equator, the latitude of the place of observation, and the altitudes of the Moon's upper and lower limbs.

A meeting was duly arranged in the home of Colonel Silius Titus specifically for the purpose of discussing the proposal, at which John Flamsteed, a young astronomer from Derby, was present as a co-opted member. He strongly criticised the *Sieur's* lunar method of finding longitude at sea on the grounds that it presupposed an accuracy in contemporary lunar and solar ephemerides and star positions very much greater than could at that time be admitted.

When news of this reached the King, he immediately assigned to Flamsteed the task of remedying the situation, and saw to it that he was provided with the basic means to do so with a minimum of delay. The choice of Greenwich as the site of Flamsteed's observatory was that of the former Gresham Professor of Astronomy, Christopher Wren, who was aware of both its scientific and architectural advantages over the alternative suggestions of Chelsea and Hyde Park. The constructional work was greatly facilitated by the fact that many of the building materials were already to hand. More bricks were brought to Greenwich from Tilbury Fort, and wood, iron and lead were obtained from a demolished gatehouse in the Tower of London where Flamsteed's patron and friend, Sir Jonas Moore, had his home. The foundations were laid on August 10, 1675, and the shell of the building completed before Christ-

mas. It became habitable on July 10, 1676, when Flamsteed moved into the small dwelling on the ground floor with his two assistants Thomas Smith (*alias* Faber) and Cuthbert Denton. The first recorded astronomical observation with a 6 foot 9 inch radius iron sextant gifted to him by Sir Jonas Moore was made from the Great Room directly above it on September 19 of the same year.

Another present from Moore was the two Tompion clocks (still preserved) in that same room, with which Flamsteed experimentally verified the values of the Equation of Time that he had previously deduced from rational principles in a Latin tract appended to John Wallis's edition of *Jeremiae Horroccii Opera Posthuma* (Londini, 1673).

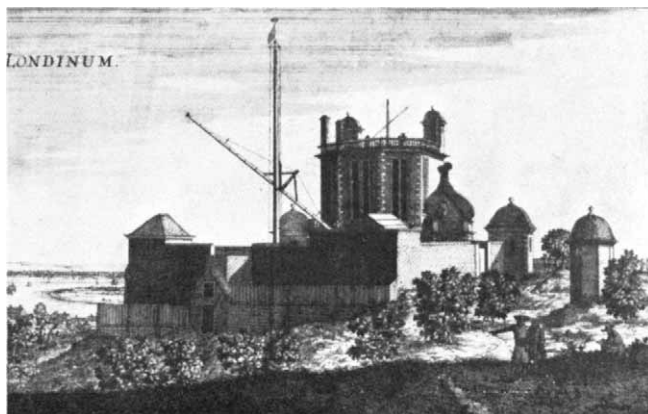
Establishing a tradition

The year 1687, remembered in the annals of science as the year of publication of Newton's *Principia*, was also the culmination of the initial phase of Flamsteed's work at Greenwich, during which he made numerous micrometric measurements of solar, lunar, and planetary diameters, as well as frequent telescopic observations of lunar occultations and small angular distances in the heavens. He examined the irregularities in the motions of the Sun, Moon and planets, established the basis of a new star catalogue, and investigated the motion of the comet of 1680. He also provided data for the theory of atmospheric refraction, and critically reviewed previous attempts to establish the value of the obliquity of the ecliptic. Insights into these and other aspects of Flamsteed's early activities at Greenwich may be gained from a study of his extensive correspondence with eminent British and foreign contemporaries—men such as Henry Oldenburg, John Collins, Jonas Moore, Giovanni Cassini, Richard Towneley, Edmond Halley, Johann Hevelius, Isaac Newton, and William Molyneux—which is currently being collated with a view to publication. To date, approximately one-quarter of this material has appeared in print, scattered throughout a number of different writings.

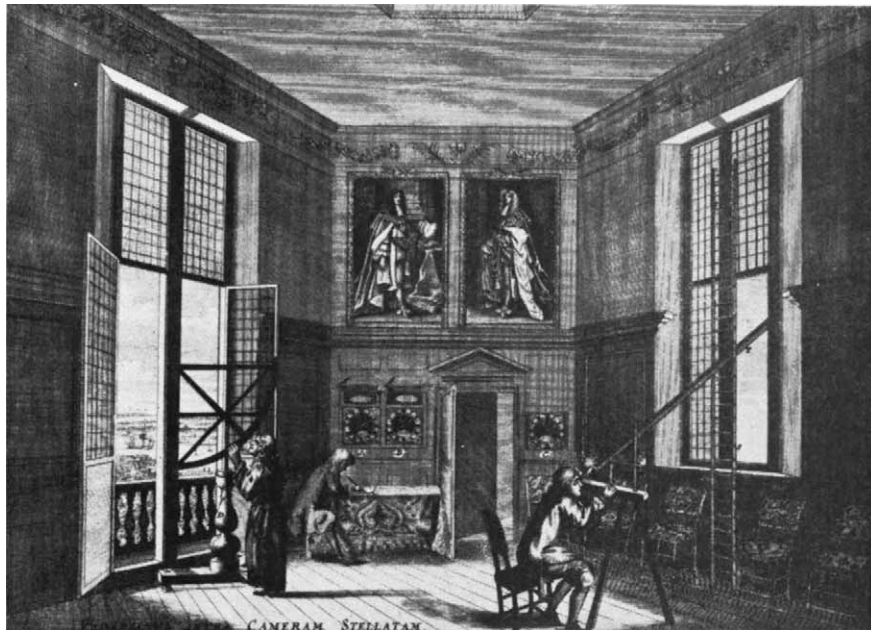
His simultaneous preoccupation with the evaluation of the Sun's parallax, the theory of cometary motion, the theory of telescope optics, the application of telescopic sights to astronomical instruments, the errors in ancient observations, the stereographic ecliptic projections of the celestial sphere, the physical constitution of the heavenly bodies, the evaluation of the precessional constant, and the theory of astronomical refraction, is discussed in my edition of *The Gresham Lectures of John Flamsteed* (Mansell, London, 1975). The publication is planned to coincide with the opening of the Greenwich Tercentenary Symposium on July 14.

The construction, by his highly skilled assistant Abraham Sharp, of a new mural arch with telescopic sights, which first became functional in October 1689, and which was

The north face of the observatory in 1676.



The Great Room, about 1676. Among the instruments on view are Tompion's year-clocks and Flamsteed's 3-foot quadrant.



paid for by Flamsteed himself with money inherited as a result of his father's death, marks the beginning of an era of intense activity in the observation of reliable star positions. Flamsteed arranged the positions into tables of transits under the name of each constellation, giving the transit times, meridional zenith distances, north polar distances, magnitude classes, and right ascensions standardised on that of some principal star in each constellation. From this information, the true equatorial and ecliptic coordinates of every star were found, and charts of the constellations drawn. To assist him in the laborious task of reducing all his raw data, Flamsteed engaged James Hodgson (who, in 1702, married Flamsteed's niece, Anna), Thomas Witty, Luke Leigh and William Bossley; he also re-engaged the services of Sharp, who had meanwhile left Greenwich and returned to his country estate in Yorkshire. He furnished Newton with hundreds of measures of refraction, and observed positions of the superior planets (Mars, Jupiter and Saturn) and the Moon.

When, in 1704, the time seemed ripe to seek financial support for publication, he allowed Newton—as newly elected President of the Royal Society—to intercede publicly on his behalf with Queen Anne's consort George, Prince of Denmark who himself proposed that Francis Robartes, Christopher Wren, and Dr John Arbuthnot should be appointed (together with any others whom the Royal Society should deem fit) to inspect Flamsteed's papers and judge when they were ready for the press. These referees coopted Francis Aston, David Gregory, and Newton. "Articles of Agreement" between these gentlemen, the publisher Awnsham Churchill, and Flamsteed himself were finalised on November 17, 1705. Under the agreement the Astronomer Royal legally bound himself to prepare and deliver "with all convenient speed . . . fair and correct copies" of his star catalogue, and to have this inserted in the first volume of the projected two-volume work.

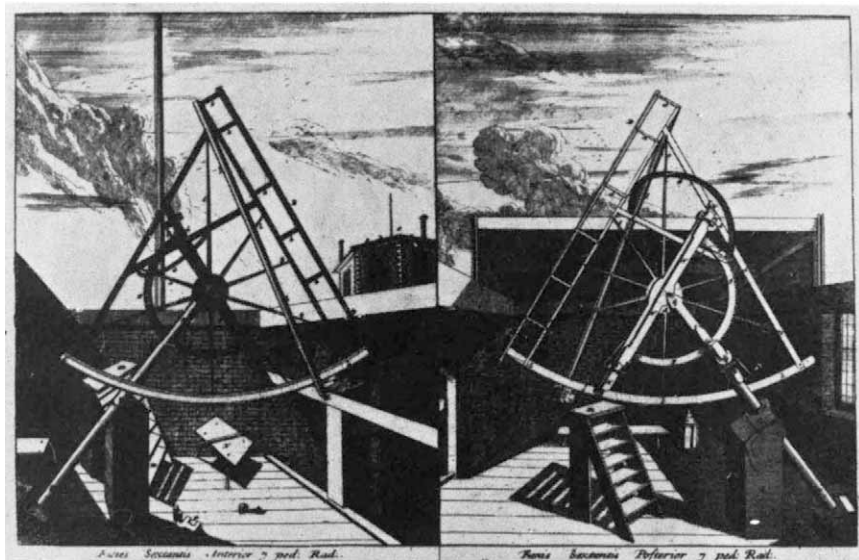
The bitter arguments which afterwards ensued between Newton and Flamsteed were primarily caused by the former's insistence that these conditions be honoured—even even after George's death on October 28, 1708—and the latter's refusal to abide by them when he saw that the printer was falling well behind in his work schedule. Flamsteed was also annoyed that Churchill was being well paid for his services whereas he, the author, got little recompense for his own labours and financial outlays in employing so many assistants.

The situation was greatly aggravated by the strong personalities of the two principal antagonists, and was finally brought to a head with Queen Anne's Warrant of December 12, 1710, which established a Board of Visitors to the Royal Observatory at Greenwich. This body consisted of the President and Members of Council of the Royal Society and others whom they should appoint. They had powers to demand from the Astronomer Royal a true and fair copy of his annual observations within six months of each subsequent year. The Board was also to report to the principal officers of HM Ordnance on any defects in the instrumentation. Flamsteed's vehement objections to Edmond Halley's appointment as editor of his observations and star catalogue were to no avail, and Halley's two-volume edition was duly published under the title *Historiae coelestis* (Londini, 1712).

Thanks to the help of Sir Robert Walpole, however, Flamsteed did manage to obtain 300 of the 500 printed copies and to burn about a quarter of what had been printed, as a "sacrifice to heavenly truth". The rest he retained for inclusion in his own three-volume *Historiae Coelestis Britannicae* (Londini, 1725) completed posthumously and without financial recompense by his devoted assistants Joseph Crosthwait and Abraham Sharp. This literary monument to Flamsteed's memory has been described by William Cudworth as occupying the same place in practical astronomy as Newton's *Principia* occupied in the theoretical aspect; and the revised catalogue of the equatorial and ecliptic coordinates of almost 3,000 stars, which comprised part of the third volume, has been praised by Francis Bailey as "the proudest production (considering the period at which it was made) of the Royal Observatory at Greenwich". The bracketed qualification in Bailey's statement is very significant, since, as he goes on to stress, the two Tompion clocks lacked any temperature compensation, analytical methods of reducing data had not yet been developed, the physical phenomena of aberration and nutation were still unknown, identifications of stars were often erroneous, the amounts of refraction were uncertain, and the temperatures and barometric pressures at the times of observation were never recorded.

Improvements in instrumentation and techniques

Halley's appointment as Flamsteed's successor on February 9, 1720, placed the former in the ideal position to resume an earlier investigation for finding longitude at sea by the



Flamsteed's 7-foot sextant; back and front views, 1676.

method of lunar distances, which serious domestic circumstances had previously forced him to abandon. The investigation involved observing the Moon at all positions in her orbit over a period of one Saros cycle of 223 lunations (18 yr 11 d)—an ambitious task for a man already in his 64th year to undertake. The prosecution of this research and the addition of some more star positions to those in Flamsteed's catalogue kept him fully occupied from the end of 1721 until shortly before his death on January 14, 1742. Four of his five vellum-bound volumes of observations are still extant, but they are in such a state of confusion that they have never been published. Only the *Tabulae Astronomicae* derived from them were published posthumously in London by John Bevis.

The most serious sources of error affecting the accuracy of all Halley's data were the poor quality of his four clocks and his failure to distinguish clearly which clock (and thus, which instrument) had been used in each observation. His energies were, however, directed primarily towards meridian transit observations of the Moon, of which he collected almost 1,500. By comparing these with the predictions of the revised lunar theory in the second edition of Newton's *Principia* (1713), he optimistically concluded that the errors in the latter were generally within $\pm 2'$, implying an uncertainty in longitude of about 60 nautical miles. In fact, this figure indicates the maximum level of accuracy which was attained; as often as not, the errors were four or five times as large and were thus quite unsuitable for use as an astronomical basis for oceanic navigation.

The state of the instruments at the Royal Observatory had always been a cause of concern both to Flamsteed and to Halley. Indeed, when the latter came to reside at Greenwich, he found himself with no major instrument and with no furniture, since everything which was not government property had been removed by Flamsteed's executors. Fortunately for him, he was given an equipment grant of £500 with which he purchased from the London instrument maker George Graham a new 5.5-foot radius transit circle and an 8-foot radius iron mural quadrant. But when the Third Astronomer Royal, James Bradley, took up office in June, 1742, he found that Flamsteed's Sextant House had become a pigeon loft and that the quadrant had jammed against the roof of the building housing it. Several very substantial alterations had to be made to both of these instruments. Bradley consequently presented a memorial to George III asking for new instruments and better buildings, and was fortunate in obtaining money for the purchase of an 8-foot radius brass mural quadrant and 40-inch radius

movable quadrant, made by Graham's protégé John Bird. He was also able to buy an excellent transit clock made by John Shelton under Graham's supervision, and to mount his own 12.5-foot radius zenith sector with which he had previously investigated stellar aberration and nutation at his Wanstead observatory near London.

Bradley's Greenwich observations, made between 1742 and 1762, consist of 13 folio volumes (of 931 pages altogether) and 2 in quarto. After his death, they were removed from the Royal Observatory by his executors and became the object of a law suit which dragged on for almost half a century before the *Astronomical Observations* were finally published in two volumes by the Clarendon Press under the respective editorships of Thomas Hornsby (1798) and Abram Robertson (1805). In the latter volume, observations by the Fourth Astronomer Royal, Nathaniel Bliss, and his assistant Charles Green, provided a connecting link between Bradley and his successors. Some years later, Friedrich Wilhelm Bessel of Königsberg was to publish his complicated reductions of all these observed star positions from their apparent to their true values. A completely new reduction by Arthur Auwers, published in Leipzig in three volumes between 1912 and 1914, is proof that Bradley's influence was still being directly felt well into the present century.

Regulating policies

Nevil Maskelyne was already concerned, even before his appointment in January 1765, as Fifth Astronomer Royal, with the predominance of contemporary continental astronomers such as Nicholas de Lacaille and Tobias Mayer over British astronomers possessing superior instrumentation. He attributed the blame largely to the manner in which Bliss's three illustrious predecessors (and, subsequently, their legal executors) had jealously guarded their respective Greenwich observations as their own exclusive property. In a memorial read to the Royal Society on June 9, 1763, he argued (unfairly, as subsequent evidence has shown) that Flamsteed's tenaciousness in withholding his observations not only from the public but also from his learned friends was responsible for a delay in Newton's establishment of the lunar theory. The Royal Society's action in setting up the Board of Visitors had facilitated increased control over the observing policy and efficiency of the Astronomer Royal, just as their action in retrieving Halley's manuscripts from his executors had prevented his whole series of 19 years' lunar observations from becoming lost.

Bradley's denial of his observations of Sirius to Maskelyne, before the latter's expedition to St Helena to observe the 1761 Transit of Venus, had forced him to rely upon Lacaille's Cape observations for 1751 and 1752 published in the *Fundamenta Astronomiae* (Paris, 1758) which seemed to favour a parallax for that star of about 8". Thus, the Royal Society had been put to unnecessary expense in purchasing a 10-foot zenith sector for the investigation of this hypothetical phenomenon, and Maskelyne had wasted much valuable time and effort in searching for it. So the lack of a clear policy regarding the legal ownership of the Greenwich observations was both weakening the impact of the Royal Observatory on the progress of astronomical science, and causing foreign scientists to think that their British colleagues were incapable of sustained and reliable observational work.

The remedy to this situation lay in the hands of those responsible for governing that important institution, the Royal Society (and, through their Board of Visitors, the Lords of the Admiralty, the Board of Ordnance, and ultimately King George III himself). What was needed, in fact, was a set of regulations clearly defining the responsibilities of the Astronomer Royal's office. Nine of these were duly drawn up by the Council of the Royal Society on November 8, 1764, for His Majesty's consideration, by which Maskelyne was soon afterwards committed to abide. Although he did not possess Bradley's data, he fell heir to his equipment and, through his former association with Bliss, knew a great deal about Bradley's observational techniques.

He wisely chose to ignore the fascinating field of cosmological speculation in which William Herschel was soon to achieve universal fame. Instead, he concentrated on determining, to the highest possible degree of precision, the equatorial coordinates of only 36 principal stars, making no attempt to follow in the footsteps of his predecessors by compiling an even more complete star catalogue. The stars which he selected for his observing programme were of great use to practising navigators taking sightings of lunar distances. His various improvements to nautical instruments, such as the Hadley quadrant, his development of the allied observational techniques, his editing of Mayer's lunar theory and tables, and his testing of marine chronometers, were all directly relevant to the needs of the British mariner. Yet he is probably best remembered by posterity on account of his foundation of the *Nautical Almanac* and his "Requisite Tables" for compensating the effects of refraction and parallax, both of which became for a century or more indispensable aids to the method of using lunar distances to find longitude at sea.

Just as important for the future of the Royal Observatory as an institution, however, were Maskelyne's successful efforts to place its affairs on a firm administrative footing. Aided by an effective and well disposed Board of Visitors, he ensured that the Board of Ordnance maintained the instruments and buildings under his care in a proper state of repair; and it was at his instigation that a Royal Warrant was issued in 1767 authorising that same Board to pay for the printing of future Greenwich observations. The regular publication of these actually began nine years later—over a century after the observatory's foundation—when the data for the years 1765 to 1769 (which he had been conscientiously submitting to the Board of Visitors at regular intervals, in accordance with one of the new regulations) were printed in a single volume. This was followed in 1787, 1799, and 1811 by three further volumes containing his observations from 1775–86, 1787–98 and 1799–1810, respectively. He managed to obtain improved salaries for his resident assistant, as well as for the four computers and one comparer on his non-residential *Nautical Almanac* staff. On the other hand, he could do little to relieve the boredom of their occupations, and

employed no fewer than 25 different assistants at the Royal Observatory during his 46-year period of office.

An era of expansion

When John Pond took up his appointment as Sixth Astronomer Royal on April 13, 1811, the Royal Observatory was a "going concern". The retrieved observations of Flamsteed and Halley were deposited under his care and the entire legacy of Bradley's, Bliss's, and Maskelyne's respective contributions to the Greenwich data was now finally in print. He also fell heir to a new instrument, Edward Troughton's 6-foot diameter mural circle, which had been ordered on Maskelyne's recommendation in 1807 by a Royal Society committee. It was destined to become a vitally important tool in his observing programme.

The great advantage of this circle, of which Pond had been using a smaller model in his own private observatory at Westbury, near Bristol, was that it enabled the observer to determine the total arc between a star (or the Sun) and the pole, without requiring a sighting of any intermediate point. It thus dispensed with the need for a plumbline—an attachment which Maskelyne had found, and demonstrated, to be very unreliable. Pond's attempt to use the new instrument to measure differences in parallax between the stars γ Draconis and α Lyrae led him to devise a novel technique involving this comparison of measurements made by direct vision with measurements made at other transits, after the star's image had been reflected off a mercury surface. The equality of the angles of incidence and reflection meant that half of the angle contained between the two readings on the circle was equal to the star's altitude; and the reading at the point of bisection indicated the horizontal point. The latitude being known, the north polar distance could then be easily determined.

A disadvantage of this method was that a whole day, or several days, had to elapse before a complete observation of the double altitude could be obtained; and the only remedy was to use two mural circles simultaneously. Thus, a new (preferably identical) instrument was required. It was duly installed at the Royal Observatory early in 1825, and two more "indefatigable, hard working & above all obedient drudges", as Pond privately classified his assistants, were hired to operate it.

Meanwhile, it had become abundantly clear that the Board of Ordnance was not the most competent body for assessing the nature and importance of the work undertaken at this observatory. Under the terms of the Act 58



John Pond, 1767–1836. Sixth Astronomer Royal, 1811–35.

George III, cap. xx (1818) "for more effectually discovering the Longitude at Sea . . ." full responsibility for the observatory's affairs was accepted by the Admiralty. The constitution of the Board of Longitude, established by the Act 12 Queen Anne, cap. xv (1714), "to provide a Publick Reward to such Person or Persons as shall Discover the Longitude at Sea", was radically revised, and Thomas Young was appointed as its paid secretary. He was also given the additional responsibilities of superintending the regular publication of the *Nautical Almanac* and looking after the regulating of chronometers.

Although the recording of the rates and the testing of the refined pieces of mechanism which were being offered for government purchase had been imposing an unwanted additional burden on Pond and his staff, this service was unquestionably of great value to the navigational aspect of the observatory's work and was to be encouraged now that it had become such a dominant feature of its activity. The Greenwich Time Ball, erected in September 1833, is symbolic of this same interest, since its function was to enable ships' captains on the Thames to check the errors and adjust the rates of their own chronometers before setting out to sea.

A vitalising force which was bringing new life into practical astronomy about this time was the Astronomical Society of London, founded on January 12, 1820. Men like Francis Baily and Stephen Groombridge, inspired by the example of Bessel, were leading its members on a campaign of meridian observation, star corrections, and reform of the *Nautical Almanac*. Other aims which were being

energetically pursued included the collation and publication of observations, the education of observers, the measurement of the length of the seconds pendulum for assisting terrestrial latitude determination, the improvement of lunar tables for finding longitude both on land and at sea, the establishment of closer working relationships with foreign astronomers, the computation of orbits, the formation of a library, and so on.

The zeal exhibited by the new society undoubtedly encouraged the Admiralty to believe that the Board of Longitude was rapidly becoming redundant and to arrange for its dissolution in 1828 by the Act 9 George IV, cap. xvi, after it had been seen to have fulfilled the function for which it had been created. Another consequence of the Society's rapidly-acquired prestige was William IV's decision on March 7, 1830 to incorporate it under Royal Charter, followed six months later by a reconstitution of the Board of Visitors which gave it equal control to the Royal Society over the affairs of the Royal Observatory. Before Pond was enforced into an early retirement in 1835 through chronic ill health and his consequent inability to cope with his alcoholic first assistant, he managed to see his catalogue of 1,113 stars and his weighty volumes of the Greenwich Observations through the press.

It was now left to his more mathematically-gifted successor, George Biddell Airy, to rescue the Royal Observatory from the general state of inefficiency in which Pond had left it, and to forge even stronger links between astronomy and related branches of the physical sciences.

Airy and after

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The Royal Greenwich Observatory of today is a vastly different institution from that founded in the seventeenth century. An original staff of two housed in one small building has risen to some 250 housed in a number of buildings scattered over more than 300 acres of grounds. Its original, primarily practical purpose of determining longitude at sea has been largely replaced by pure research. If the observatory's history is traced with these various changes in mind, it becomes apparent that the transition period from the early style to the modern occurred during the nineteenth century when Airy was Astronomer Royal.

WHEN Pond resigned as Astronomer Royal in 1835, he left the Royal Observatory in some state of disorder and it was apparent that a confirmed disciplinarian with a strong sense of organisation would be needed to reform the workings of the observatory. This might almost read as a specification of G. B. Airy—a man who seemed born to authority in the astronomical world. Before the age of 30, Airy had been appointed to the Lucasian Professorship at Cambridge, once held by Newton, and had resigned it in favour of the Plumian Professorship at the same university. The latter post entailed superintendence of the University Observatory, initially with no extra salary, but with accommodation provided. (This led to the contemporary Cambridge jest that the university authorities "gave to Airy nothing, a local habitation and a name".) But Airy used the opportunity to

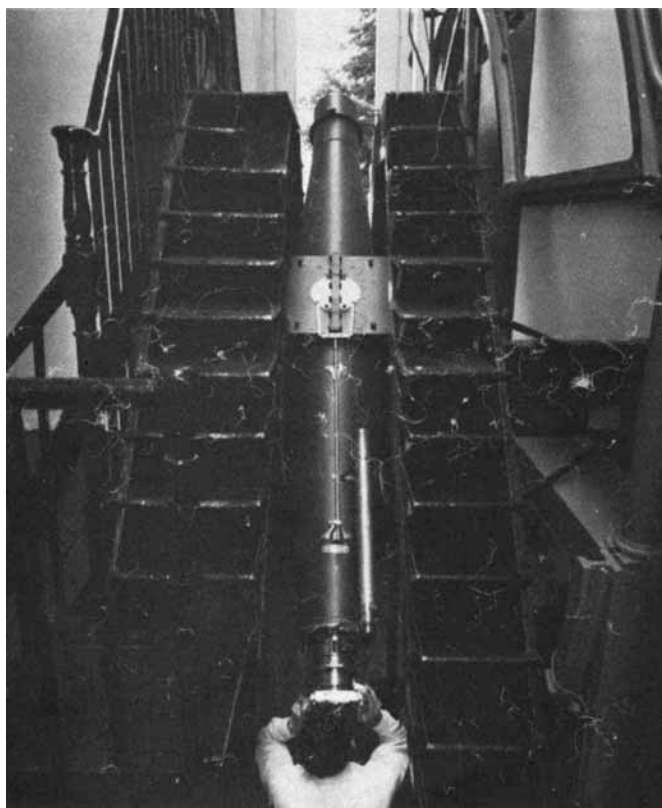
work out his own ideas on how an observatory should be run: a demonstration of organisational ability that was not lost on his peers when the need to find a replacement for Pond arose.

Airy possessed, indeed, a total faith in the value of proper organisation: it was a guiding principle in all aspects of his life. As his friend De Morgan observed: "If Airy wiped his pen on a piece of blotting-paper he would duly endorse the blotting-paper with the date and particulars of its use, and file it away amongst his papers"¹. Allied with this was a considerable mechanical ingenuity, so that organisation often took the form of a mechanical contrivance: "... the whole of the Observatory was full of his inventions—doors which shut by contrivances of his own, arrangements for holding papers, for making clocks go simultaneously, for regulating pendulums, for arranging garden beds, for keeping planks from twisting, for every conceivable thing from the greatest to the smallest"².

Reorganising the observatory and staff

These characteristics of Airy proved to be decisive in the development of the Royal Observatory. The observatory had built up during its history a fine reputation for accurate observational work. But it had also gained a reputation for being none too rapid in publishing usable results from these observations. Airy applauded the former aspect of the observatory's work, but deplored the latter (which he believed to be a failing of English astronomy in general).

"In England an observer conceives that he has done everything when he has made an observation. He thinks that



Airy's Transit Circle on the Prime Meridian.

merely noting the passage of a star over one wire and its bisection by another, is all that can be expected from him; and that the use of a Table of logarithms or anything beyond the very first stage of reductions, ought to be left to others"³.

At the British Association meeting of 1833, before the question of his Greenwich appointment arose, Airy proposed that all Greenwich observations of the planets made between 1750 and 1830 should be reduced to a uniform system. After taking over as Astronomer Royal, he made a similar proposal with regard to the lunar observations accumulated over the same period. Both proposals were approved, and attracted government aid for the hire of temporary computing assistance to carry out the work. The planetary computations were published in 1846 and the lunar reductions two years later. From that time on, the reduction of Greenwich observations has remained in step with the acquired observations: Airy established what has become a well known Royal Observatory tradition.

By the time Airy retired in 1881, the status of Greenwich as a producer of accurate positional data on a continuing basis was acknowledged throughout the world. The vast amount of data could be handled efficiently as a result of Airy's introduction of the methods of mass production into astronomy. He analysed the equations used in reducing positional observations into a series of steps in elementary mathematics that could be, and were, applied by schoolboys. He added checking procedures at each stage to prevent errors creeping in, and the whole process of reduction generally went like clockwork.

In terms of work efficiency, Airy's reorganisation of the Royal Observatory clearly benefited the observatory greatly, but the effect on the staff was much less happy. The typical work rota of an assistant under Airy would run thus: day 1,

on duty for 21 hours, observing continuously; day 2, 2-3 hours computing; day 3, full day's computing followed by night duty; day 4, 2-3 hours computing.

In the early days of Airy's reign, the assistants concerned with meteorology and magnetism had even harder schedules, for they were required, when on duty, to read their instruments at two-hourly intervals throughout the day and night. The assistants, for their part, regarded the lot of the temporary computers as even harder, for the latter not only worked long hours on tasks of a most boring nature: they had absolutely no guarantee of continued employment. At the end of the nineteenth century, one of the assistants at Greenwich had this to say: "So, too, [Airy's] regulation of his subordinates was, especially in his earlier days, despotic in the extreme—despotic to an extent which would scarcely be tolerated in the present day, and which was the cause of not a little serious suffering to some of his staff The unfortunate boys who carried out the computations of the great lunar reductions were kept at their desks from eight in the morning till eight at night, without the slightest intermission, except an hour at midday"⁴.

This criticism is not, however, altogether fair to Airy. Although he certainly demanded a Victorian dedication to hard work and obedience to authority, he was not entirely unreasonable. When it could be done without loss of efficiency, he was quite prepared to mitigate harsh conditions of service. For example, he converted the meteorological and magnetic instruments to self-registering forms—thus relieving the assistants—as soon as the opportunity offered. (In fact, he made Greenwich one of the first institutions to have self-recording devices for these purposes.)

Along with some improvement of staff conditions, the nineteenth century also saw the beginnings of the present hierarchical organisation of staff at the observatory. When Airy took over, the Astronomer Royal traditionally had a different background and education from the assistants. Airy's first step was to change this by appointing a First Assistant with similar qualifications to his own, who could act as a trustworthy second-in-command. In September 1835 he explained to the Admiralty: "I consider it as most important that the First Assistant should be a man of respectable rank in society. This alone can give a hope of security against the danger of being corrupted with money by makers of chronometers, and of losing authority over the subordinate assistants by having recourse to them for assistance in disgraceful difficulties: both which things, according to common report, have occurred at the Royal Observatory".



George Airy, 1801-92. Seventh Astronomer Royal, 1835-81.



Airy crossing the Great Court at the observatory; an 1839 picture.

In the 1870s, following on the general reorganisation of entrance requirements to the Civil Service, assistants at Greenwich came to be appointed by competitive examination. The calibre of staff rose rapidly, and, as a direct consequence, the Admiralty agreed to a modification of the staff hierarchy. The First Assistant—now relabelled the Chief Assistant—still came immediately below the Astronomer Royal, but there were now two tiers of assistants below him (First Class and Second Class). Under Airy's successor, W. H. M. Christie, two Chief Assistants were appointed, and the link between Astronomer Royal and Chief Assistant became very close. Whereas previously the Astronomer Royal had always been appointed from outside the Royal Observatory, it now became customary to choose someone who had had experience at the observatory as Chief Assistant. Christie, himself, was the first of such appointees.

One of the major complaints of assistants under Airy had concerned their inadequate salaries. This continued to be a source of contention under Christie, though the salary scales gradually improved. The problem was that equivalent ranks elsewhere in the Civil Service could earn almost twice as much as a Greenwich assistant; and the more educated recruits now coming forward felt the difference strongly. There was a similar problem at the computer level. By the end of the nineteenth century, school leavers were finding desk jobs relatively easy to obtain. As a result, the turnover in junior staff at the observatory became rapid. In a report to the Board of Visitors in 1901, Christie noted that: "Within the last five months one-third of the whole Staff of Computers have left the Observatory for other posts and have had to be replaced by boys new to the work". The poor salary scales and career prospects for most staff remained a problem at the observatory well into the twentieth century.

New equipment and standard time

Along with changes in organisation, the nineteenth century saw great innovations in the instrumentation used at Greenwich. Airy, with his pronounced interest in engineering design, had the need for new equipment in mind from the beginning. He did not act, however, until the back log of computational work was out of the way. Then, in a few years round 1850, he replaced much of the existing equipment with new instrumentation designed by himself. What was to become the best known of these instruments—the Airy transit circle—came into service in 1851. The last measurements with it were made over a century later, in

1954, by which time it had been used for over three-quarters of a million observations.

The transit circle, like most of the other instruments introduced by Airy, was intended to carry out the traditional aim of the observatory—accurate positional measurement. But Airy also introduced, at a slightly later date, a refracting telescope, large by the standards of the day, that allowed observations of a more general kind. By the second half of the nineteenth century, it was becoming clear that the research front in astronomy was gradually moving away from the old system of precise positional measurement in the meridian to an interest in the physical nature of the objects studied. Airy realised, reluctantly, that if the Royal Observatory were to remain the premier observing centre in the country it must move with the times. By the early 1870s he had decided that a fundamental addition must be made to the work of the observatory.

"The tendency of late discoveries and consequent discussions in astronomy has been, not to withdraw attention from the exact departments of astronomy, but to add greatly to public interest in those which are less severely definite. And this has become so strong, that I think it may well be a subject of consideration by the Board of Visitors whether observations bearing upon some of those trains of discovery should not be included in the ordinary system of the Royal Observatory

The character of the Observatory would be somewhat changed by this innovation, but not, as I imagine, in a direction to which any objection can be made. It would become, *pro tanto*, a physical Observatory; and possibly in time its operations might be extended still further in a physical direction" (from the Report to the Board of Visitors, 1872).

Airy's rather suspicious attitude towards new types of observation was not evident in his successor. Indeed, Christie brought into operation a new building—larger than any of the old buildings—devoted especially to physical observations. By the end of the nineteenth century, photographic and spectroscopic work had become a routine part of the observatory's programme.

When Airy took command at Greenwich he found that a large proportion of staff time was actually devoted neither to the acquisition of observations, nor to their reduction. Instead, it was spent on checking marine chronometers for the Navy. In his autobiography, he noted that: "In the inferior departments of the Admiralty, especially in the Hydrographic Office (then represented by Captain Beaufort) with which I was principally connected, the Observatory was considered rather as a place for managing Government

chronometers than as a place of science. The preceding First Assistant had kept a book of letter references, and I found that out of 840 letters, 820 related to Government chronometers only."

Airy battled against this overemphasis (as he saw it) on non-astronomical activities. He succeeded in antagonising the Admiralty, but was only partly successful in his campaign to cut back the amount of chronometer work required. The proportion of staff time devoted to this did decrease slightly down the years, but the number of chronometers on hand for checking actually rose appreciably. In the later years of the century, during Christie's period of office as Astronomer Royal, naval expansion led to a further rapid growth in the number of chronometers under test. In fact, Christie used this increase as a basis for his plea for new buildings at Greenwich.

Airy's objection was purely to the volume of routine work involved: he regarded the improvement of chronometers and the dissemination of accurate time signals as certainly falling under the aegis of the Royal Observatory. One of the major advances in dissemination of Greenwich time was a result of Airy's initiative. By the middle of the nineteenth century, the electric telegraph was coming into use on the new railway network in Britain. As a result of negotiations with the nearest line—the South Eastern Railway—Airy arranged for time signals from Greenwich to be transmitted into central London and thence out along the telegraphs on the principal railway lines. Soon after this service was established, Airy was approached by the Post Office, who also wished to receive time signals from the Royal Observatory. By the 1870s, Greenwich time signals were being distributed by the Post Office to a number of towns throughout the British Isles. They were being used even more extensively to regulate post office and railway clocks in all parts of the country.

For much of the nineteenth century, Britain had no uniform standard of time keeping. London followed Greenwich time, but areas elsewhere typically used their own local times. As travel, especially by rail, became commoner, this began to give rise to problems. For example, according to *Bradshaw* a trip from London to the west of England took six and a half hours, whereas the return journey took seven and a half hours. In fact, the train took the same time in both directions: the apparent difference arose because of the change in longitude, and, therefore, in local time. Greenwich Mean Time (GMT) did not finally become the official time throughout most of the country until 1880. Even then, Ireland was excluded: GMT only came into use there in 1916.

Just as the lack of standard time raised problems within Britain, so the lack of international agreement on time and longitude standards raised problems between countries. Different nations took their zero longitude and time from different places. The Paris Observatory, for example, provided the standard time and longitude for France. In 1884, following various preliminary exchanges, delegates from 27 countries attended a conference in Washington to try to agree on a generally acceptable prime meridian. After much bargaining (and consideration of a list of candidates that included Jerusalem, Rome and the great pyramid at Giza), Greenwich was accepted as the zero point for measurement of longitude, and GMT consequently became the basic time. Many factors were involved in this choice—including strong support for Greenwich from the American hosts—but one fundamental reason, as indicated by the choice of the Airy transit circle for the exact zero point, was the observatory's unassailable reputation for positional work.

In a sense, the selection of Greenwich as the prime meridian can be seen as a final summing of the practical purpose for which the observatory was originally founded. From the latter part of the nineteenth century onwards, the main expansion of the observatory's work has been into fields of

fundamental importance in astronomy; but there have been only occasional practical applications in those fields. At the same time, the functions of the Astronomer Royal have become restricted more closely to the field of astronomy. Airy, for much of his life, acted as a general adviser on science to the Government of the day. He was consulted over a range of matters that had nothing to do with astronomy—sewers, gas meters, lighthouses, railway gauges. Airy's only known poem was written when he was under attack for advising that Charles Babbage should not receive further funding from the Government. (Babbage needed more money to complete his "difference engine"—a distant forerunner of the modern computer.) In one verse of the poem Airy explains:

"Now the whole is made clear by a short explanation:
When Babbage for money made fresh application
The Treasury (most likely) could do nothing wiser
Than refer it to Airy, their only adviser."

This was the point: Airy was one of the very few professional scientists employed by the Government, and he was therefore appealed to whenever scientific knowledge was needed. By the end of the nineteenth century the number of professionals available had grown considerably: the Astronomer Royal was no longer a unique figure.

It would be wrong, however, to end on a descending note, for, as the general advisory powers of the Astronomer Royal decreased, so his significance in the astronomical world at large rose. The latter part of the nineteenth century saw a major development of international scientific cooperation and organisation. The characteristics required for successful leadership in this field proved to be very much those required of a successful Astronomer Royal. Consequently, Airy in his later years, and Christie, increasingly, became involved in the international organisation of astronomy. That trend is one that has continued with growing momentum in the twentieth century.

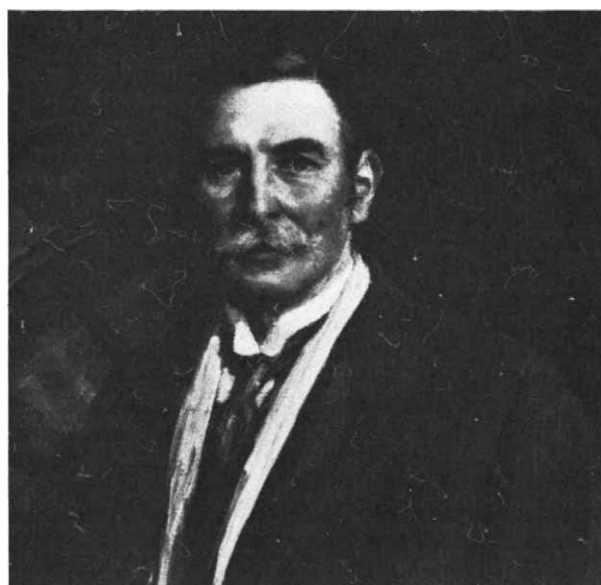
¹ Maunder, E. W., *The Royal Observatory, Greenwich*, 116, (Religious Tract Society, London, 1900).

² Stuart, J., *Reminiscences*, 159 (Chiswick Press, London, 1911).

³ Airy, G. B., *Second Report of the British Association*, 184 (1832).

⁴ Maunder, E. W., *ibid.*, 117.

⁵ Airy, W., *Autobiography of Sir George Biddell Airy*, 124 (Cambridge University Press, London, 1896).



William Christie, 1845–1922. Eighth Astronomer Royal, 1881–1910 [Courtesy: E. W. Christie].

Greenwich Observatory in the twentieth century

Alan Hunter

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Alan Hunter traces the history and future of observational astrophysics at the RGO in the twentieth century and the continuing role of the RGO in positional astronomy.

Kicking and screaming . . . ?

At the time that the Royal Greenwich Observatory (RGO) entered its third century things were beginning to change in astronomy. By the end of the nineteenth century the development of the dry photographic plate and the application of the spectroscope to the stars and nebulae had begun a revolution that was to steer optical astronomy steadily away from traditional positional work towards astrophysics. It was a change of emphasis that at first was not shared at all at Greenwich, and is not shared fully even now.

To the extent that the process of dispersing light reduces its intensity, and particularly also because of interest in fainter and more distant objects, the need grew for telescopes of bigger aperture on sites with better observing conditions than could be got in Britain, or indeed in northern Europe. The ideal situation was in the USA, where in California latitude, and therefore climate, were favourable, where mountains existed that projected above the cloud instead of into it, and where, by the turn of the century, the engineering skills that made big telescopes possible created also the big personal and institutional fortunes from which they could be financed. From the time the 60-inch telescope was erected on Mt Wilson in 1908, European astronomy lost its lead. It has never regained it.

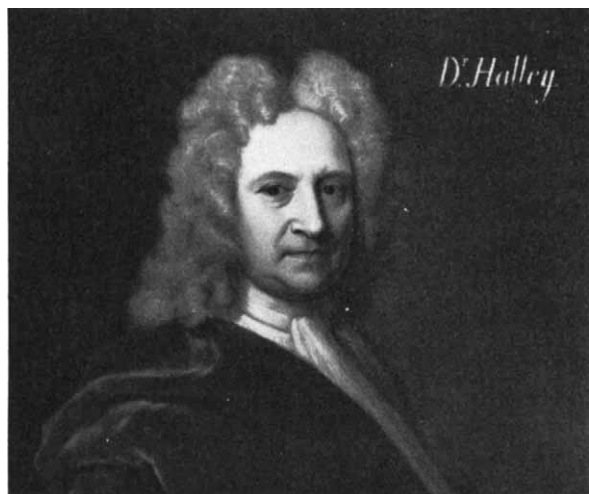
By the same token, the RGO also lost its scientific position as unchallenged leader of world astronomy: not because the observatory had changed but because astronomy had changed and the observatory had not. Paradoxically, the international adoption in 1884 of Greenwich longitude as zero, and of Greenwich Mean Time as the basis of the world time zones, was concurrently making its name as familiar among laymen around the world as it had been for two centuries among astronomers. But there is astronomy and astronomy. The

unconscious arrogance that long ago christened meridian astronomy as 'fundamental' has given way in modern times to an equally insensitive myopia that equates optical astronomy with astrophysics. This has been well shown by recent criticisms^{1,2} of UK optical astronomy and astronomers. It is a pity that these criticisms have been expressed with such intemperance as to be largely discounted; for much of what the critics have to say has substance. British astronomy has grown lopsided for want of access to big optical telescopes, and the defect is only now beginning to be put right.

Much of the trouble lay in past history. For reasons that go back to the circumstances of its foundation by Charles II, the observatory was, between 1818 and 1955, administered by the British Admiralty. Now Lord Rothschild's customer-contractor relationship is new only in enunciation, not in concept. For wholly understandable reasons the Admiralty was willing to pay only for what it wanted, and it got what it paid for: an observatory well designed to carry out positional astronomy, but ill equipped to explore the physical universe. In the circumstances, successive Astronomers Royal have done well to wheedle out of private donors a succession of what were at the time big telescopes (a 26-inch refractor in 1897, a 30-inch reflector in 1898, a 36-inch reflector in 1934), and still better to persuade the Admiralty to allow their use on activities that did not advance navigation one whit, nor improve the precision of the time service an iota. But these activities remained peripheral: the emphasis stayed on positional astronomy. Meanwhile observational astrophysics remained still-born in universities in the UK while the bright youngsters followed Eddington (a Chief Assistant at Greenwich) into theory and, later, Lovell and Ryle into the new science of radio astronomy. Those who felt they must observe or perish emigrated to the USA or to South Africa, where the best of them demonstrated that it was lack of opportunity, not lack of capacity, that held back observational astrophysics in the UK.

Bigger apertures

The construction of the Isaac Newton Telescope, bedevilled as it was throughout by planning delays and stop-go economics, could go only part way to a solution. The proposal³ came at a time when success in a hard-fought war had resulted in a honeymoon between British science and politics that begot the money for the telescope but not the change in Whitehall thinking that would have allowed its erection on foreign soil. Later criticism⁴ of the siting of the telescope at Herstmonceux has been largely misdirected. Speaking with the benefit of hindsight, the critics ignore both the transformation that has occurred in administrative thinking since the money was voted in 1946 and the transformation in logistics that has been imported by the jet engine. It was not until after such international bodies as CERN and ESRO had shown that UK investment in science abroad was not unthinkable that any hope existed for support in British government circles for the idea of running big telescopes overseas. Private beneficence had shown the way. The Radcliffe Trustees erected, on what was in 1938 a good site near Pretoria, a 74-inch reflector that has, since it was commissioned in 1948, done first-class pioneering work in observational astrophysics in the Southern Hemisphere. True, the telescope was seriously under-endowed, but a series of administrative devices channelled public money into its operation, in return for observing time, until eventually the



Edmond Halley, 1656–1742. Second Astronomer Royal, 1720–42 [Courtesy: National Portrait Gallery].

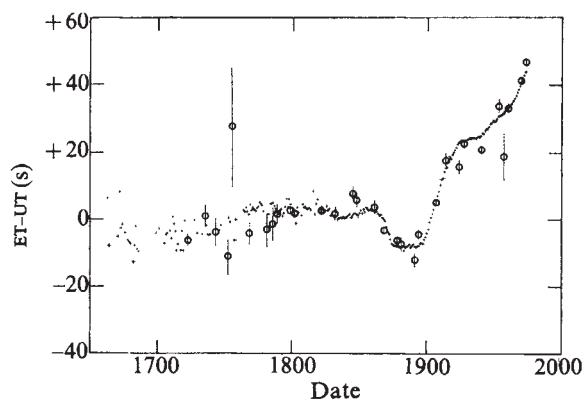


Fig. 1 Three centuries of the rotating Earth. The quantity plotted against date is the difference between Ephemeris Time (ET), the uniform time that reconciles planetary observations with gravitational theory, and Universal Time (UT), the irregular time shown by the rotating Earth. Crosses mark observations of the Moon; circles with s.e. bars mark transits of Mercury across the Sun's disk⁷.

South African government purchased it from the Trustees in 1974. That its turret is now rising again on one of the world's finest astronomical sites at Sutherland, some 200 miles north-east of Cape Town, as part of a South African observatory run jointly with the UK, ensures British access for the foreseeable future to a large telescope of proven capacity in the Southern Hemisphere.

New administration

The crucial factor in associating the RGO with these developments was the formation of the Science Research Council in 1965 and its immediate assumption of administrative control over the observatory as well as over the Royal Observatory at Edinburgh. At the same time it inherited also the grant-giving functions of the old Department of Scientific and Industrial Research. For the first time in history all UK astronomy came under the same direction. There are obvious dangers in monopoly, but they are so obvious that, foreseen, they can be avoided. Hard things have been said—continue to be said—about the somewhat elephantine committee structure of the SRC and the mountains of paper it generates. But it is a structure in which, if the will exists, bureaucracy can be controlled by active committee chairmen, and monopoly can be moderated by external assessors. And within it there exists for the first time a possibility that UK astronomy can be put on its feet.

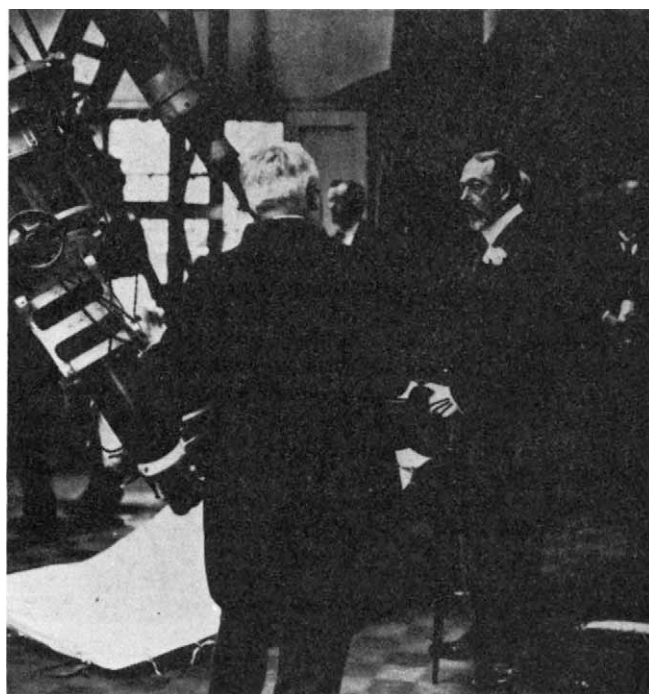
The way was shown, in what was seen at the time by some as blind optimism but can with hindsight be described as inspired faith, by Sir Richard Woolley while the observatory was still run by the Admiralty. Inheriting from his predecessor an establishment decimated by the war, with its competence even for positional work diminished by fifteen years of enforced disuse, and with no foreseeable capacity for work on faint objects, he proceeded to build up research astrophysics and instrumentation. His complement of staff had to share the post-war rundown of the defence departments, so he was forced to get his manpower largely from internal regrouping. He pruned to the bone activities in meridian astronomy, in solar physics, in the Nautical Almanac Office and in the time department—all without diminishing output to a point that threatened survival of the older work. Armed with vacancies produced by natural wastage, he began recruiting young scientists, many of them attracted into astronomy through attendance at highly successful summer vacation courses he had earlier instituted at Herstmonceux. Recognising the impracticability of trying to do effective extragalactic work under Sussex skies, even with an aperture of 98 inches in prospect, he set them to work on galactic objects, whether of intrinsic interest

themselves or as part of a wider study of the kinematics and physics of the Galaxy. Simultaneously he sounded out among US and Commonwealth astronomers the prospects for collaborative operation of a large telescope overseas. A favourable response came from Australia, and the resulting 150-inch reflector was inaugurated by Prince Charles in October 1974. It is now being commissioned under the direction of Professor E. J. Wampler, by a team of observers in which Woolley's band of youngsters is prominently represented.

The northern sky

With access assured to the 74-inch telescope at Sutherland and the 150-inch telescope at Siding Spring, UK astronomers are well catered for in the Southern Hemisphere. As seen from these telescopes the galactic centre passes overhead, and the Magellanic Clouds, our nearest external galaxies, are well visible. But there are insistent reasons why UK optical observers can never be satisfied with telescopes in the Southern Hemisphere alone; notably because of the continuing need to monitor optically that range of northern sources that their radio colleagues have been so prominent in investigating. There was therefore immediate and strong support (not least from radio astronomers) when Professor H. A. Brück, Astronomer Royal for Scotland, suggested that the UK should establish a large telescope on a favourable spot in the Northern Hemisphere. The proposal has now grown into the Northern Hemisphere Observatory (NHO) project, still mainly British but with some participation by other European countries, which has received interim approval by the SRC and is now at the active planning stage. The role of the RGO in this project has been a subject of controversy for years, but earlier disagreements were resolved by a positive SRC decision in 1974 that the design, procurement and eventual operation of the NHO shall be a prime responsibility of RGO. That this must be achieved without permanent increase in the complement of staff will mean a major restructuring of the observatory that is now under way. Since the national and international functions of the RGO are, as we have seen above, already on the edge of understaffing, this must mean a reduction of effort in research. Such a reduction must not, of course, be carried too far, for

King George V inspects the 28-inch equatorial telescope, 26th July 1925.



there could be no more certain recipe for producing bad telescopes and worse ancillaries than to have them designed without the constant surveillance of active observers. But so long as a basic competence is retained within the RGO in the major fields in which the new telescopes are to be used, the diversion of effort will be made without complaint in the belief that in no other way can UK observational astrophysics be set on the road to recovery.

The erratic Earth

With its steps turned firmly towards a future that can now include extragalactic studies, the RGO can look back with some satisfaction on its achievements in this century. That these have been primarily in positional astronomy is, it is true, a feature that has not been of its own choosing. Nor is it one that calls for apology. Many astrophysical advances depend heavily on a substratum of astrometric knowledge that must be kept updated if we are not to experience in the next century an imbalance no less regrettable than that in this. It is arguable that the observatory's most lasting contributions to astronomy reside in the long series of catalogues that started with the *Historia Coelestis* and continue today with modern meridian positions to an accuracy Flamsteed never dreamed of. Delambre, in his history of eighteenth century astronomy, claimed that if the whole of recorded science were destroyed in some cataclysm, except for the observations made at Greenwich by Bradley and Maskelyne, it would be possible to reconstruct from these records almost the whole edifice of astronomy as it was then known. Half a century later, the American astronomer Simon Newcomb repeated the statement in almost the same words in respect of the series of Greenwich lunar observations up to his own time. If a similar claim (of course for positional astronomy alone) cannot be maintained now, a century later, it is only because of the incorporation of positional work in the Southern Hemisphere into the body of astronomy; and Greenwich has been closely associated with that through its connection with the Royal Observatory at the Cape of Good Hope.

Space forbids the tracing here of more than one line of work to which Greenwich astronomers have made major contributions in this century. If we choose this to be the investigation of the Earth's rotation, the topic will take us from the intricacies of celestial mechanics to the form of the time signals that enter every home in the country.

The last Astronomer Royal to confine his own work at Greenwich almost exclusively to positional astronomy was Spencer Jones. In a real sense, he can be said to have completed the lunar work started by Flamsteed 250 years earlier. Success in accounting fully for all the finer details of the Moon's

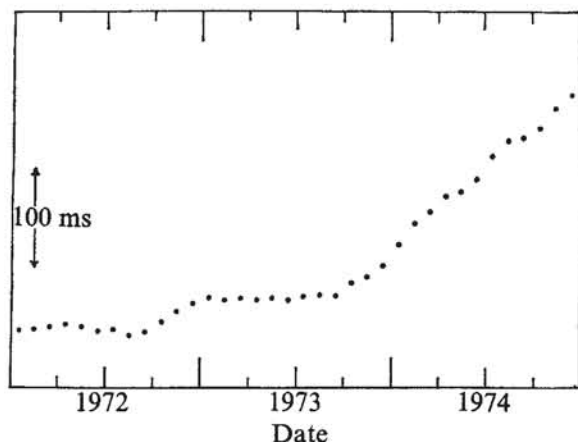


Fig. 2 Three years of the rotating Earth. The quantity plotted against date is the difference between Earth Time (corrected for seasonal variation and polar motion) and Atomic Time. An arbitrary slope has been removed to show better the half-millisecond reduction in the length of the day that became apparent in October 1973.

motion by gravitational theory had eluded the nineteenth century practitioners of celestial mechanics. The tables constructed by Burckhardt contained errors that had to be corrected in Hansen's. Hansen's in turn were superseded by Brown's; and yet there existed divergences from observation that needed reconciliation by unexplained empirical terms. It was left to Spencer Jones by a classical investigation⁵ in 1939 into the motions of Sun, Moon and inner planets to show that the fluctuations observed in the positions of all these bodies could be simultaneously explained by abandoning the belief that the Earth was rotating uniformly. Earlier, it had been tacitly assumed that the time variable in gravitational theory could be satisfactorily represented in terms of the angular rotation of the Earth, as measured by stellar observations and counted by clocks kept in consonance with those observations. But if the time intervals shown as equal by the rotating Earth were not equal in fact, small wonder that the positions of the Moon and planets predicted from gravitational theory failed to accord with observation. In effect, the positions observed at one time were being compared with the predictions made for another. By his brilliant and exhaustive investigation, Spencer Jones was able to show that the irregular fluctuations in the mean longitudes of the bodies studied are strictly proportional to their mean motions, as also are the secular accelerations. He attributed these effects respectively to irregular changes in the Earth's moment of inertia and to a long-period slowing of the Earth's rotation due to tidal friction.

Flamsteed had used his Tompion clocks to verify that, to navigational accuracy, the Earth was rotating uniformly. Spencer Jones used the observations of his predecessors at Greenwich (not indeed back to Flamsteed but to Bradley) to demonstrate that to a higher accuracy it was not. It was a coincidence that the horological means of direct detection of the rotational irregularity were at hand. During the Second World War the oscillating quartz crystal displaced the vibrating pendulum as the basic element of the most accurate clocks, and soon⁶ it had been established that the length of the day fluctuated seasonally. In turn the quartz oscillator (now obtainable for the mantelshelf and even for the wrist) has more recently given way to the atomic beam resonator. Time can be picked from this with such precision that both short and long term fluctuations in the Earth's rotation can be directly observed. This has precipitated a change in the definition of the second, which since 1967 is no longer 1/86,400 of a day but the interval comprising 9,192,631,770 periods of the radiation emitted between two hyperfine levels in the ground state of the caesium-133 atom.

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It is now necessary, therefore, to distinguish two time scales: the uniform time provided by atomic clocks and required by radio engineers and physicists who need accurate frequencies, and the old Universal Time still linked to the rotating Earth, still needed by astronomers, navigators, surveyors and space scientists, and still derived from observations of the stars. The two scales are so closely the same that there can be no question of radiating different time signals; the scope for confusion would be immense. The signals emitted define, as closely as can be achieved, intervals of International Atomic Time; and the seconds pulses continuously radiated by stations such as MSF are by international agreement relabelled when necessary to keep closely in general accord with the Earth by the insertion (or deletion) of a leap second. The long term trend (see Fig. 1) currently requires one or two leap seconds to be inserted annually, though it must be remarked that the most recent evidence (see Fig. 2) indicates that in October 1973 the Earth speeded up its rate of rotation for the first time since monitoring against atomic time began in 1955. For the few purposes requiring better contemporaneous accuracy in epoch than 0.7 second, the signals are coded; for the most precise purposes, corrections are published in retard.

Alterations in the length of the day amounting to a milli-second or so may sound small enough for the man in the street to ignore, but of course if they are systematic in trend they will accumulate till they become important. Atomic time cannot be extrapolated backwards before the days of atomic clocks, but if it measures the independent variable needed to reconcile observations of the heavenly bodies with gravitational theory it becomes possible to go back to the earliest records. Figure 1 shows that in a man's lifetime the Earth may slow enough to make atomic time unsuitable for navigation. Records of Greek eclipses in the pre-Christian era show that the Earth has lost several hours as a timekeeper since then. It is a practical

necessity, therefore, to keep the epoch of atomic time close to Earth Time; and this necessity is reflected in the decision to lengthen the sixth pip of the familiar Greenwich time signal. Now that it is required, once or twice a year, to broadcast seven pulses, it becomes desirable to avoid confusion by distinguishing the business pip from the others. The half-second pip is unmistakable amongst its tenth-second fellows: it is the beginning of this lengthened signal that marks the time.

In all this work Greenwich has taken a prominent part internationally under H. M. Smith, whose service to the RGO and to world timekeeping has spanned the interval between the 0.1 second accuracy of the pre-war time signals and the submicrosecond precision of today. Perhaps the most important distinguishably Greenwich contribution has been made on the observational side, where the Photographic Zenith Tube designed by D. S. Perfect has, since 1954, shown consistently less observational scatter than similar instruments elsewhere and still determines time from a night's observation of the stars to ± 4 ms. As noted above, such observations are no longer used directly in controlling the radiated time signals; they contribute to the international decision as to when leap seconds should be inserted in labelling the emitted train. Their main scientific interest, by an inversion not uncommon in the interplay between science and technology, is now chiefly geophysical, in monitoring the rotation of the Earth, rather than astronomical, in using the rotation to determine the time of day. Airy, Maskelyne, Halley, Flamsteed even, would have been delighted.

¹ Burbidge, G., *Nature*, **239**, 117 (1972).

² Strittmatter, P. A., *Nature*, **239**, 475 (1972).

³ Plaskett, H. H., *Mon. Not. R. Astr. Soc.*, **106**, 80 (1946).

⁴ Sadler, D. H., *The Observatory*, **66**, 380 (1946).

⁵ *Nature*, **239**, 121 (1972).

⁶ Spencer Jones, H., *Mon. Not. R. Astr. Soc.*, **99**, 541 (1939).

⁷ Finch, H. F., *Mon. Not. R. Astr. Soc.*, **110**, 3 (1950).

⁸ Morrison, L. V., and Ward, C. G., *Mon. Not. R. Astr. Soc.* (in the press).

Sidelights on the Royal Observatory

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The name of the RGO today is synonymous with stability and respectability. But, as this article shows, such a position has not been achieved without a few qualms.

"INSTRUCTIONS for Cuthbert Denton one of the Lab^{rs} to y^e Office of His Maj^{ty} Ordnance to goe downe to Greenwich to be employed in His Maj^{ty} Service or else where as shall be found fitt at y^e Observatory or other His Maj^{ty} Service.

You are hereby required to Repare to Greenwich Hill afores^d & accordingly there to Obey & follow such Ord^{rs} & Directions that shall be given you by y^e s^d Sir Jonas Moore either to repare to Labour at Woolwich, Deptford or Else where as he shall think fit".

The above instructions, included in a warrant¹ signed by Thomas Chicheley on February 10, 1675–76, contain the first reference to the Royal Observatory's industrial staff.

Cuthbert (no doubt the silly, surly labourer mentioned by John Flamsteed) had a multitude of duties, including helping Thomas Smith, the first Astronomer Royal's sole official assistant, with observations, when he usually managed to do something wrong.

Opportunities to avoid work were rare but he did succeed

in achieving this on more than one occasion. In a letter² to Sir Jonas Moore, Surveyor-General of Ordnance, dated January 17, 1677 Flamsteed complained that "On Monday last I gave Cuthbert leave to goe to London to receive some Monyes due to him, with charge to return before night, which he promised but came not home till Wednesday morning, so I lost two of y^e best & clearest nights we have had this good while".

Apart from his astronomical work, Flamsteed did sometimes take part in scientific experiments of the day. One such, to determine the speed of sound, was arranged as follows. A mortar was to be fired from the beacon on Shooters Hill and the interval between flash and report timed by a party 'of gentlemen' at the Royal Observatory. Preparations included a servant (no doubt Cuthbert) on the roof of the Great Room (now Flamsteed House) provided with a musket with which it was intended to signal to the gunner on Shooters Hill, exactly three miles away. One can only assume that all this took place at dusk so that there would be some chance of seeing the flash. In the event, the gunner failed to see or hear the musket fired and the whole experiment was in danger of being wrecked at the crucial moment by unexpected gunnery practice by ships on the Thames, only half a mile distant, but eventually everything

turned out satisfactorily and Flamsteed concluded that sound travelled three miles "in about $14\frac{1}{2}$ seconds".

Denton, who suffered from "the gripes", may or may not have sympathised with Flamsteed over the latter's ill-health, so often the subject of comment.

"1678 July 20. Began to be ill; an hectick fever and hick kough

August 6. Went to London where S^r Ch. Scarborough prescribed to me & by God's mercy recovered".

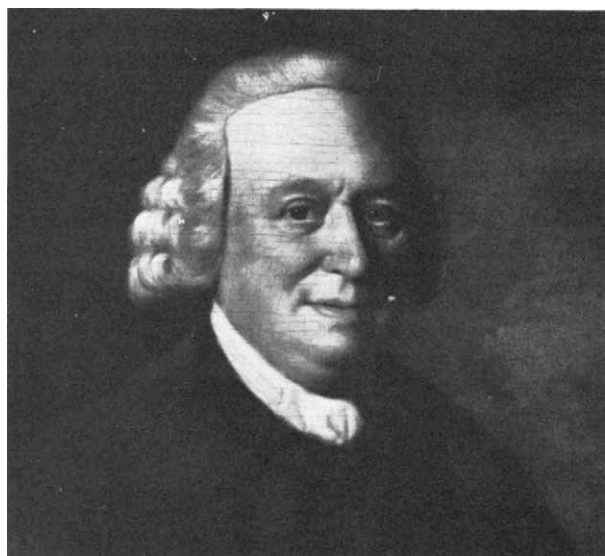
In his later years the Astronomer Royal took to home-made cures and popular nostrums "for y^e Gout"³.

"Take half a dozen Gallons of malt wort into which put 12 Handfulls of Alehoofe or Ground Ivy, 3 handfulls of pine tops, 3 handfulls of sage, 6 jags of Steel". One was advised in due course to drink a pint of this alarming brew every morning "& at spring and fall take a bottle of Daffy's Elixir".

Links with Paris

During Nevil Maskelyne's term of office (1765–1811) there occurred an incident with far-reaching consequences. The Director of the Royal Observatory in Paris, Cassini de Thury, (1714–84) transmitted in October 1783, through the French Ambassador, a memoir to the Crown⁴ in which he "set forth the great advantages that would accrue to astronomy, by carrying a series of triangles from the neighbourhood of London to Dover, there to be connected with those already executed in France, by which combined operations the relative situations of the most famous observatories in Europe, Greenwich and Paris, would be more accurately ascertained than they are at present". The first and speedy result of this was the foundation of what became the Ordnance Survey; another was that Sir Joseph Banks, President of the Royal Society, forwarded the memoir to Maskelyne together with the request for an answer to Cassini's remarks about the relative positions of the Royal Observatories.

Cassini claimed that the longitude and latitude of differences between Greenwich and Paris were out of agreement by 11' and 15", respectively, and that, as the French triangulation had been carried as far as the tower of Dover Castle, the 11' of longitude must all be between Greenwich and Dover.



Nevil Maskelyne, 1732–1811. Fifth Astronomer Royal, 1765–1811.



War damage to the Altazimuth Pavilion (foreground), 1940.

Maskelyne's detailed reply⁵, read to the Royal Society on February 22, 1787, was in the form of a lengthy discussion in which he examined Bradley's and his own observations, stated that the latitude difference between Greenwich and Paris was $2^{\circ} 38' 26''$, and that the "error cannot be more than $3''$ or $4''$ " rather than $15''$ supposed by Cassini. He was right; today's value is $2^{\circ} 38' 27''$.

Regarding the discrepancy in longitude, he pointed out that Bradley made the longitude of Paris $9^{\text{m}} 20'$ East of Greenwich while he had inferred $9^{\text{m}} 25'$.

Since then he had examined many observations made at the Royal Observatories and by Messier (at the Hotel de Cluny). Considering all available reliable observations, he concluded that the difference of meridians was " $9^{\text{m}} 20'$, being within a very few seconds of the truth". Again he was correct; the present value is $9^{\text{m}} 20.91'$ (equivalent to $9^{\text{m}} 20.93'$ from Bradley's transit telescope, some 19 feet West of the Prime Meridian).

An intriguing incident arising from all this is revealed in a small private notebook⁶ belonging to Joseph Lindley, who was Maskelyne's assistant from July 1781 to September 1786. The Astronomer Royal despatched Lindley by post chaise and cross-Channel packet to Paris and back between September 20 and October 3, 1785 to determine the longitude difference by chronometric means, carrying eight of John Arnold's watches—four large, including the King's watch and four small, including Lindley's own. Comparisons were made at the Paris Observatory after a journey of 6 d 10 h, after which Lindley made the return journey without delay but evidently not without incident, as he noted about his own property that "This watch happen'd some accident coming home at Boulogne . . . but it went exceeding well till then".

He noted "Oct 3 d, Arriv'd at the Observatory at 10^{h} PM", when final comparisons were made. The longitude difference deduced from the large watches came to $9^{\text{m}} 20.5'$ and from the small ones $9^{\text{m}} 18.3'$. Giving double weight to the large time-pieces, the mean was $9^{\text{m}} 19.8'E$, a remarkably good result.

This may well have been the first cross-Channel longitude determination by chronometers and Maskelyne may have been seriously pioneering what was to become a standard procedure until the introduction of the electric telegraph in the next century. No official mention of the operation can be found in the Royal Greenwich Observatory archives.

It is interesting that the entries in the Dunsink Observatory Transit Book, 1788, for miscellaneous longitude determinations include the following, by Henry Ussher,

Andrews Professor 1785–90 (communicated to me by Professor P. A. Wayman).

“Longitude of Paris by Mr Arnold’s watch.

1st journey 9^m 20^s, 2nd journey 9^m 15^s and 9^m 16^s, 3rd journey 9^m 21^s”

These are, unfortunately, undated and otherwise unexplained.

Time keeping

In the next century one of the many undertakings connected with “extraneous business” was G. B. Airy’s involvement in the construction of the clock and bells for the New Palace of Westminster.

As early as June 20, 1846 he received a letter⁷ from Viscount Canning, then First Commissioner of Woods and Works, who wrote, “It is of importance that the Clock which is to be placed in the clock-tower of the New Houses of Parliament should be the very best which British science and skill can supply. The Commissioners of Woods, etc., are most anxious to ensure this, and they would therefore be glad to have the advantage of your opinion as to the best means of doing so.”

Airy’s ten-page reply on June 22 involved him in 15 years of correspondence and meetings.

Towards the end of this period he was trying to settle the question of the bells to sound the chimes. Although tone-deaf, he had, with others, bravely undertaken to arrange the chimes and musical tones of the bells. The chime arrangement chosen was that of St Mary’s church, Cambridge, but difficulties arose when the notes of the bells had to be defined and Airy resorted to employing professional musicians (at a guinea a visit) to listen to the bells, which were struck with hammers of tin and gutta percha recommended by the Astronomer Royal. The musicians then wrote down their versions of the musical tones. Unfortunately, with varying styles of notation their conclusions bore little resemblance to each other.

Advised to consult the engineer responsible for the chiming mechanism, Airy on June 8, 1860, reported that “I do not think that Mr Jabez James is at all musical”. Having fought his way through some of his difficulties, he presented his suggestions to W. F. Cowper, Chief Commissioner of Works, who replied on June 26, with disconcerting frankness, “I really cannot undertake to decide the point about the notes myself and I have no-one in my office with a musical ear”.

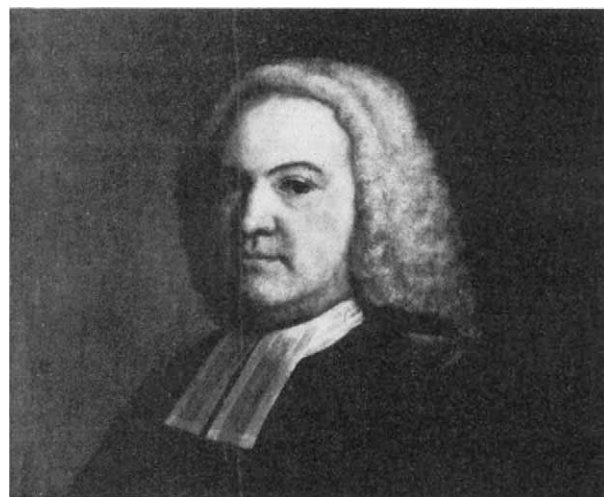
In frustration Airy then turned to William Pole, Professor of Engineering, who was also a Bachelor of Music, and on August 6, wrote, “I would also beg you to stand in the stage above the bells when a Quarter or Hour is struck, that you may hear the tremendous beating, which exceeds anything that I ever heard”.

In his reply (posted the same day!) Pole hit the nail on the head: “It is only at a certain distance that the chief note of a bell makes itself predominant . . . and hence I do not wonder at any difficulty found in determining the note when *close to the bell*, the chief note being smothered and confused with a multitude of other sounds, harmonic & otherwise”.

Pole, armed with tuning forks and other equipment, then withdrew a suitable distance and completed the task.

It must have been with a feeling of distinct relief that Airy eventually received a letter from the First Commissioner of Her Majesty’s Works thanking him “for the trouble you have taken with regard to the bells which has led to so satisfactory a result”.

One final touch; on February 19, 1861, Airy wrote to Pole, “I have not yet succeeded in getting from the Department of Works the £1 which is due to you on the account of the Westminster bells . . . I enclose therefore a cheque and will take my chance of getting it returned.”



Nathaniel Bliss, 1700–64. Fourth Astronomer Royal, 1762–64 [Courtesy: M. N. Dix-Hamilton].

Observational work

It should not be thought that the Astronomer Royal was the only person at the Royal Observatory occasionally to find himself in difficulties.

Observing aids for staff working with the larger telescopes often provided unsuspected hazards. The observing couch in use with the 28-inch refractor consisted of a large frame, angled at about 30°, supporting a large and reasonably comfortable mattress on which the observer reclined. It is remembered that once a senior member of the staff was observing double stars (entailing, if seeing was poor, fairly long intervals waiting for the definition of close images to improve sufficiently for them to be observed with precision) when he drifted off to sleep in the observing position on the couch. Although he had ceased to work, the driving motor of the telescope had not and he was rudely awakened by the eyepiece plus several tons of telescope pressing remorselessly into his eye socket and pinning him to the mattress. His panic-stricken yells for help are alleged to have been heard a mile away. When he pulled himself together, it was, in fact, an easy matter to extricate himself with his eye intact—but not his reputation!

Another chair, for the 26-inch refractor, was devised by the Royal Observatory workshop to improve the comfort and efficiency of the observer. This comprised an open rectangular frame on universal castors, on which was mounted a conventional folding deckchair. But the canvas was rarely inspected and on occasions parted without warning. The victim, suspended by his knees like a trapeze artiste, cracked his head on the floor (a foot or so below) with stunning force.

On another occasion, this device, carelessly adjusted, unexpectedly folded up and effectively trapped a somewhat unpopular member of the staff for about half an hour before being extricated by the night-watchman on his rounds.

Wartime difficulties

Moving nearer to the present, early in 1940, the Admiralty decided to form an Internal Defence Unit and declare the Royal Observatory a “vulnerable point”. An area of about 200 yards radius from the outbuildings was to be patrolled by members of the staff to prevent possible sabotage and to this end there shortly arrived six Lee-Enfield rifles (Mark I, made shortly before the Boer War) together with several hundred rounds of ammunition 0.303-inch Mark VI made in 1911) and two 0.455-inch calibre revolvers with appropriate,

but technically illegal, ammunition. A unit was formed, ranks generally corresponding to Civil Service grades, and guards mounted nightly to guard the establishment.

The first mounting of the guard, which took place inside the Porter's Lodge, is still vivid in the memories of those present. The first to load his rifle performed the operation in a soldierly manner, holding the weapon at the high port, and applied the safety catch. No. 2 guard, watching in admiration, remarked that the rifle was cocked, whereupon No. 1 said, "Oh, is it?", released the safety catch and pressed the trigger. The 1911 ammunition responded well; in the restricted space of the lodge (about 10 feet square) the detonation seemed tremendous. The bullet passed some six inches in front of No. 2's nose, two inches over No. 3's head and produced a four-inch cavity in the wall. No. 4 guard, who had his back to these proceedings, disappeared from the lodge so rapidly that none of the others saw him move.

This was the first war damage caused to the Royal Observatory, but when heavy night bombing began in September 1940, the buildings were twice hit and evasive action became instinctive. By that time the Internal Defence Unit had been incorporated in the Greenwich Park section of the 16th Company, 25th County of London Home Guard, with which it continued to serve until stand-down.

On one memorable night of intensive bombing, the duty squad had, during a brief lull, emerged to investigate the situation and strayed some way from cover. When some

20 yards from the comparative safety of the observatory buildings, they heard the approach of what was currently known as a "Molotov breadbasket". This unpleasant device consisted of a canister, containing many smallish but effective high explosive bombs, which opened at a fairly low altitude and deposited its contents over a restricted area. Audibly, it gave the impression of a tramcar descending from the sky.

In the rush for cover, the leading member of the squad reached the very narrow door of the building a few yards ahead of his companions. Being of a cautious nature, he had slung his rifle across his back instead of over one shoulder; he negotiated the door satisfactorily but the rifle did not and brought him to an abrupt halt, clawing the air in an endeavour to reach safety. The rest of the squad, in total darkness, hit him squarely in the small of the back, projected him and the rifle (now bent at 45 degrees) through the door and charged across his prostrate form. Incredibly, no noticeable damage was sustained by the human door mat, although for some time he bore a handsome bruise in the form of a hob-nailed boot imprint high up between his shoulder blades.

¹ RGO Manuscripts, Reference 35, f98.

² Ibid., Reference 36, f51.

³ Ibid., Reference 7, f179.

⁴ Phil. Trans. LXXV, 389 (1785).

⁵ Ibid., LXXVII, 151 et seq. (1787).

⁶ RGO Manuscripts, Reference 303.

⁷ RGO Manuscripts, Reference 1178, Section 1 (all other abstracts in this episode from the same source).

Astronomy three hundred years ago

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In the late 17th and early 18th centuries astronomers were coming to grips with phenomena ranging from comets to cosmology. This article describes how some of them set about solving the problems that emerged.

When beggars die, there are no comets seen;
The heavens themselves blaze forth the death of princes.
Julius Caesar, Act II Scene 2

ON the night of December 12, 1680, a hen in Rome gave an extraordinarily loud cackle and laid her first egg. It was not an ordinary egg, but unusually large and marked with the image of a comet. A few days later a brilliant comet appeared in the evening sky with a tail stretching almost from the horizon to the zenith. The egg was promptly interpreted as a portent for the celestial visitor, and reports of the egg travelled rapidly; in Germany and France broadsides appeared with the wonderful and fearsome news. Even the French Academy of Sciences felt obliged to comment on the prodigy¹.

The secretary of the academy, Bernard de Fontenelle, took a lighter view of the matter, and in a comedy entitled *La Comète* set a dialogue between a countess and an astrologer who is preparing a horoscope for a forthcoming wedding:

COUNTESS: What's happened at Rome that's more terrible than here?

ASTROLOGER: A comet—you will never guess what *kind* of comet: a comet in an egg.

COUNTESS: A comet in an egg! I'll never eat eggs again!

The contrast between the popular superstitions and the increasing rational understanding of comets was never more striking than in that decade. The Great Comet had been

discovered in the morning sky in mid-November 1680. It was soon under scrutiny by the leading French observer, J.-D. Cassini, and by the English Astronomer Royal, John Flamsteed. Soon the comet vanished in the morning twilight, but in December it reappeared in the evening sky. These observations were eventually used by Isaac Newton in his *Principia* (1687), where he showed that this comet moved in a parabolic trajectory around the Sun.

Today we would class the "blazing star" of 1680 as a Sun-grazing comet, similar to Comet Ikeya-Seki of 1965. The orbit of such a comet is extremely elongated, so that the approach and the regress can almost be approximated by straight lines. The apparent path, as viewed from a moving Earth, is rather convoluted, particularly because the comet accelerates rapidly as it approaches the Sun.

From his careful series of observations on the comet of 1680, Flamsteed concluded, contrary to the general opinion, that the morning and evening appearances were of the same comet before and after it reached perihelion. Newton, who also observed its course, agreed with other astronomers that there had been two comets. Through an intermediary Newton⁵ wrote to Flamsteed that "I am further suspicious that ye Comets of November and December wch Mr Flamstead accounts one & ye same Comet were two different ones . . . If they were but one Comet, it's motion was thrice accelerated & retarded."

A few years later, when Newton made the intuitive leap to include comets within the scope of universal gravitation, the vexing apparent triple acceleration promptly fell into place, and Newton saw that the November and December appearances necessarily belonged to the same comet. In a further analysis of this comet, Newton reported in his *Principia* that a remarkable comet had appeared four times at equal intervals of 575 years beginning with the month of September in the

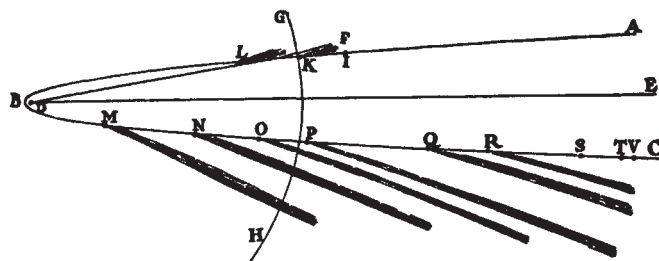


Fig. 1 The parabolic orbit of the sungrazing Comet of 1680, as it appeared in Newton's *Principia*.

year Julius Caesar was killed. Newton and his younger colleague Edmund Halley believed that the Great Comet of 1680 had been the same one as seen in 1106 and 531 AD, and 44 BC. This conclusion was in fact false, and the Great Comet of 1680 had a much longer period.

Within a few years, however, Halley correctly analysed the periodicity of another bright comet from the same decade, the comet of 1682. His laborious search in the historical records disclosed that notable comets had appeared in intervals of about 75 years, and working on this assumption he predicted (in 1705) that the comet should return late in 1758. In a later edition of his cometary tables he added: "If it should return according to our prediction about the year 1758, impartial posterity will not refuse to acknowledge that this was first discovered by an Englishman". Indeed, the comet now bears his name and is expected to return again in 1986.

Planets, conjunctions and Satellites

PRINCE: Saturn and Venus this year in conjunction!

What says th'almanac to that?

POINS: And look whether the fiery Trigon his man be not lipping to his master's old tables, his note-book, his counsel-keeper.

Henry IV, Part 2, Act II, Scene 4

Shakespeare's fleeting reference to a planetary conjunction and the fiery trigon⁴, made at the turn of the 17th century, probably evoked in the minds of his audience the widespread dire predictions associated with the great conjunction of Jupiter and Saturn in 1583. Such a conjunction, when the slow-moving Jupiter overtakes the still slower Saturn, is the rarest astrological phenomenon among the planets visible to the naked eye, occurring every 20 years. Hence astrologers seized on these events as particularly significant portents.

Johannes Kepler knew that the conjunction of 1604 was the first in several centuries to take place within one of the three zodiacal signs associated with fire (the fiery trigon); for this reason, he believed, a brilliant nova subsequently appeared near the triple planetary configuration of Saturn, Jupiter and Mars. Such speculations added fuel to the interest in astrology, which gained numerous adherents in the 17th century. Eloquent practitioners, such as William Lilly, issued one pamphlet or almanac after another. Among them were *Englands propheticall Merline, foretelling . . . untill 1663 the actions depending upon the influence of the conjunction of Saturn and Jupiter 1642*. Lilly's success in predicting the London fire of 1666 got him in no little trouble with the House of Commons who, suspecting that he may have done something to implement his prophecy, summoned him "to answer such questions as were then and there asked him." (Lilly got off free after the cross-examination.)

Even John Flamsteed felt obliged to cast a horoscope for laying the foundation stone of the Greenwich Observatory August 10, 1675 at 3.14 p.m., although he added the Latin line *Risum teneatis Amici* ("You may keep laughing, my friends"). Naturally the Astronomer Royal took particular pains to observe the great conjunction in 1683, but whereas

the English populace was wondering what the conjunction portended, Flamsteed himself was curious about the deficiencies in the planetary tables. In spite of the enormous improvements made with the introduction of Kepler's *Rudolphine Tables* in 1627, Jupiter and Saturn still did not move precisely as predicted.

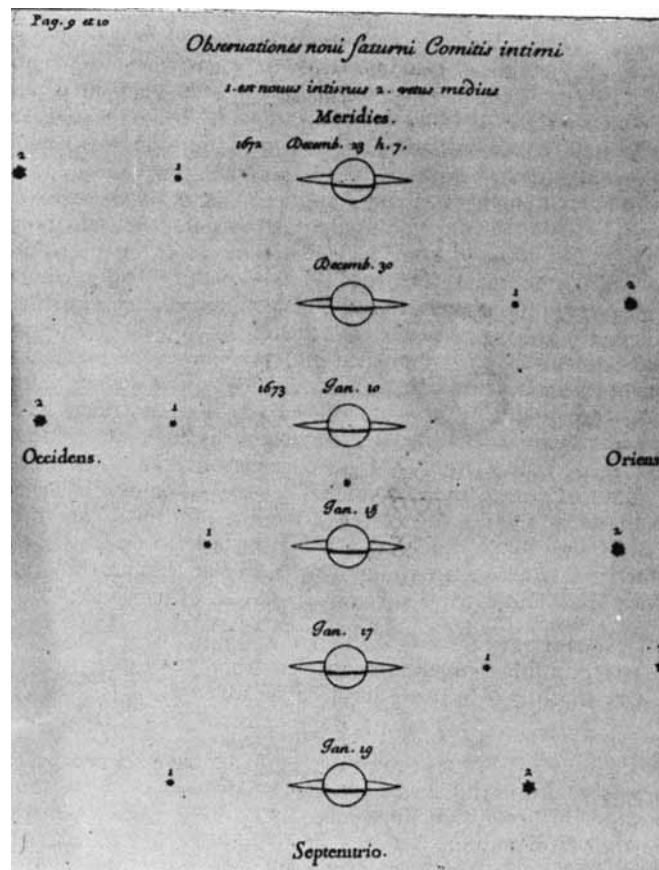
The reason, at least qualitatively, was not long in coming to light. At the end of 1684 Newton wrote to Flamsteed⁶:

"[Saturn] so oft as he is in conjunction with Jupiter ought (by reason of Jupiters action upon him) to run beyond his orbit about one or two of ye suns semidiameters or a little more & almost all the rest of his motion to run as much or more within it. Perhaps that might be ye ground of Keplers defining it too little. But I would gladly know if you ever observed Saturn to err considerably from Keplers tables about ye time of his conjunction with Jupiter."

Flamsteed replied that he found Saturn about 27' slower than Kepler's numbers, and Jupiter about 14' or 15' swifter. Newton's early insight into the interaction between Jupiter and Saturn is quite remarkable, but a quantitative solution did not emerge until a century later with the work of Lagrange and Laplace. They found a rhythmic perturbation with a period of about 900 years, resulting in a maximum deviation for Saturn of 51'.

Newton also asked Flamsteed about the satellites of these planets. He wrote: "You seem to insinuate as if Saturn had not yet any more Satellits [*sic*] than one discovered by Hugenius. I would be glad to know if it be so". The brightest Saturnian satellite, Titan, which Newton refers to, had been found by Christiaan Huygens in 1655. At that time the outstanding mystery of Saturn was the pair of curious appendages sometimes seen on opposite sides of the planet. A few years later Huygens correctly interpreted these as a thin, flat ring. It was a *tour de*

Fig. 2 Cassini announced the discovery of two new satellites of Saturn in 1673: here is shown a new one together with the brighter satellite Titan found in 1755 by Huygens. In 1684 Cassini discovered two more companions of Saturn. (Permission of Harvard College Library.)



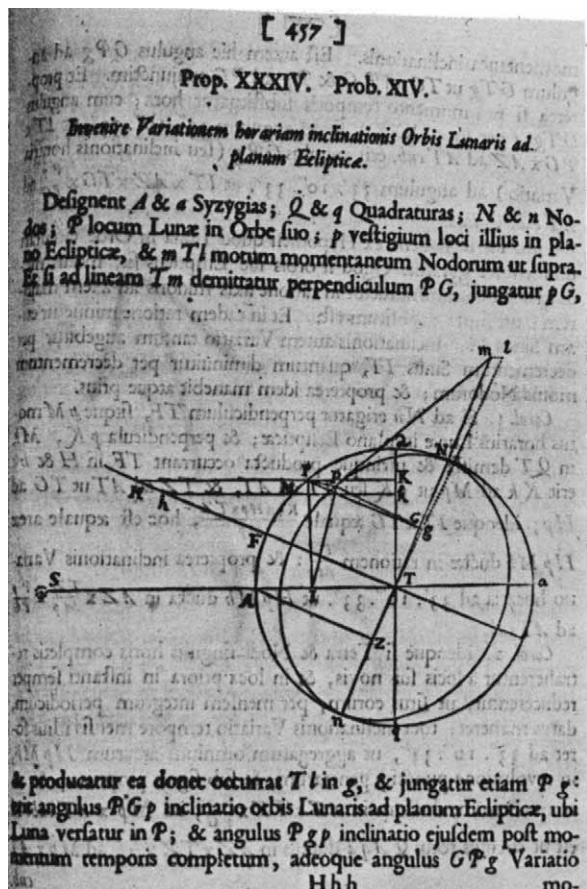


Fig. 3 Newton's demonstration of the variable inclination of the moon's orbit, an effect first observed in 1570 by Tycho Brahe. From his *Philosophiæ naturalis principia mathematica*, 1687. (Permission of Harvard College Library.)

force of theoretical reasoning, for Huygens arrived at his explanation when the rings were edge-on and hence invisible. He secured the priority of his discovery by publishing a scrambled Latin anagram, and then waited until the rings began to open up again before decoding his claim.

Following Huygens the next important work on Saturn was done by J.-D. Cassini², an Italian astronomer who had been brought to France by Louis XIV to become the leading intellectual spirit at the newly founded Paris Observatory. In September 1671 Cassini discovered the second satellite of Saturn, Iapetus, and in 1672 he observed the third, Rhea. In 1675 he discovered that Saturn's ring is subdivided into two parts separated by a narrow band, now called Cassini's division. Cassini's long telescopes, made by the best lens makers of the day, were apparently much superior to any available in England, and thus Flamsteed had written in 1684 to Newton that "I have no opinion of ye supernumerary satellites [*sic*] of Saturn". Earlier in that year Cassini discovered two more Saturnian satellites, Tethys and Dione, but nearly a century was to elapse before other new satellites were found in the Solar System (in 1781, when Herschel discovered Uranus and two of its satellites).

O, swear not by the moon, th' inconstant moon,
That monthly changes in her circled orb,
Lest that thy love prove likewise variable.

Romeo and Juliet, Act II, Scene 2

Predictions of eclipses, curiously enough, have tended to be simpler and thus more accurate than predictions of the Moon's position in general. Eclipses necessarily take place when the Earth, Moon and Sun fall on a straight line, where the gravitational interactions are least complicated. Because of the eclipses,

astronomers from antiquity onwards have concentrated on developing a theory for lunar motion that would be accurate at times of new and full Moon.

In 1590, the lack of a reasonable lunar theory for other parts of the orbit landed the Danish observer Tycho Brahe in a peculiar situation⁶. In preparation for a forthcoming lunar eclipse, Tycho observed the Moon several days beforehand, that is, in a part of the orbit not then ordinarily kept under surveillance. Unknown to Tycho and all previous astronomers, the Moon's position deviates by an easily observed 40' at this point by comparison with its traditionally accepted orbit; oblivious of this fact, Tycho apparently misjudged the beginning of the eclipse (and was perhaps caught indoors eating his dinner as it began). Puzzled and irritated by this state of affairs, Tycho began systematic observations in other parts of the orbit and promptly discovered the 40' 'variation'.

One of Newton's great triumphs was to explain the lunar 'variation' as a consequence of the three-body system involving the Sun as well as the Earth and Moon. The gravitational theory predicted still further perturbations in the Moon's orbit, and in the 1690s Newton became increasingly eager for lunar observations to test his theory. Flamsteed, whose determinations of lunar positions were the most accurate yet made, sent hundreds of observations to Newton⁸.

Flamsteed apparently hoped to construct his own empirical lunar model, however, and he was openly critical of parts of Newton's work. Newton, never one to accept criticism graciously, became rankled and eventually cut off the correspondence. Later, as President of the Royal Society, Newton attempted to exact his revenge by forcing the Astronomer Royal to publish his observations in a form dictated by Newton.

Fig. 4 A rare surviving page from the pirated Halley-Newton edition of Flamsteed's observations. Flamsteed recaptured all but 60 of these copies and destroyed this section. (Permission of Harvard College Library.)

ORDO	STELLARUM DENOMINATIO	Ascensio Recta	Distantia a Polo Bor.	Longitudo	Latitudo	Variet. An. R.	Variet. Di. & F.	P. 200
1	In Cornu praecelsa	20 46 00	69 17 15	12 04 58 B	18 20 22 27	6		
2	In Cornu praecelsa	21 25 45	71 15 25	26 48 15	9 01 26 B	52	04 23 00	7 6
3	In Cornu praecelsa	22 51 15	74 36 55	26 49 04	5 22 59 B	57	38 22 00	7 6
4	In Cornu praecelsa	24 08 30	72 14 45	28 31 00	7 08 58 B	58	16 21 41	4
5	In Cornu praecelsa	24 33 30	70 45 35	29 37 59	8 28 16 B	58	35 21 41	3
6	In Cornu praecelsa	24 39 45	67 57 45	29 04 20	10 57 12 B	59	10 21 30	10
7	In Cornu praecelsa	25 07 00	72 41 15	29 10 57	5 25 12 B	58	05 21 31	6
8	In Cornu praecelsa	25 11 00	67 36 35	29 12 15	10 47 47 B	50	12 21 31	5
9	In Cornu praecelsa	26 23 30	65 25 05	29 14 13	13 31 52 B	59	58 21 37	6 7
10	In Cornu praecelsa	27 19 30	62 28 15	29 12 12	12 04 07 B	60	05 21 38	6



Isaac Newton, the frontispiece to the third edition of the *Principia* (1726).

Flamsteed, a careful observer, resisted these demands; he knew that his derived positions of the Moon were no more accurate than the coordinates of the stars that provided his basic reference system, and he was determined to improve the star positions before publishing his results. Thus he handed over his star catalogue in a sealed envelope, with the idea that an improved version would be printed only at the end of the work. Newton, in close collusion with Halley, opened the envelope and sent the preliminary catalogue to the printer. What was essentially a pirated edition of Flamsteed's *Historia coelestis* was published in 1712. When political fortunes changed, Flamsteed captured all the undistributed sheets, destroyed them and arranged for his own version, which was published posthumously in 1725.

In spite of the bitter quarrel between Flamsteed and Newton, their respective researches provided for a much better lunar orbit. A test soon became possible; after half a century during which no total eclipse had been observable in England, two came in close succession. Edmond Halley issued an impressive broadside showing both the observed path of the 1715 eclipse and that predicted for 1724. An interesting commentary appears on a copy of this broadside now preserved at The Library Company of Philadelphia. James Logan, a notable colonial American book collector, went to Windsor to observe from a site just within the predicted path of totality. Although clouded out, he noted that it did not get dark and hence Halley's prediction was in error. Observers who had gone somewhat further from London did succeed in seeing the eclipse. From the annotation on the chart it is obvious that Logan's opinion of Halley was not improved when the latter made no attempt to correct his tables to conform with the reports of what had actually been seen. But although Halley's predictions were still somewhat imprecise, they in fact offered a substantial improvement over previous calculations.

O, learn'd indeed were that astronomer
That knew the stars as I his characters . . .

Cymbeline, Act III, Scene 3

In the year that Shakespeare wrote *Cymbeline* (1610), Galileo Galilei turned his newly perfected telescope towards the Milky Way and discovered a host of new and previously unexpected

stars. Although Galileo's discovery in principle opened up the new field of sidereal astronomy, its potentialities were scarcely exploited in the 17th century. The new exceptions, however, are fascinating.

In 1668 the Scottish mathematician James Gregory proposed that if stars were distant suns, the comparative distances of the Sun and a star could be estimated from a measurement of their relative brightnesses⁷. The determination of such photometric distances posed the practical problem of comparing two bodies very different in brightness. Gregory suggested that Jupiter



Fig. 5 Halley's broadside showing the observed path of the 1715 solar eclipse and the predicted path of the 1724 eclipse (1715). (Permission of Harvard College Library.)

could be used as an intermediary, since a geometrical calculation (together with an assumption about the reflectivity of Jupiter) allowed a simple comparison of the brightnesses of the Sun and Jupiter. In this way Gregory calculated that Sirius was 83,190 times as far away from the Earth as was the Sun.

Newton, who owned one of the rare copies of Gregory's book, discussed the problem in his "System of the World", which was originally intended as the concluding part of his *Principia*. Written in 1685, it was published posthumously in 1728. In article 57, on the distance of the stars, Newton enlarged Gregory's estimate, placing a first magnitude star 100,000 times further away than Saturn, or about 950,000 solar distances

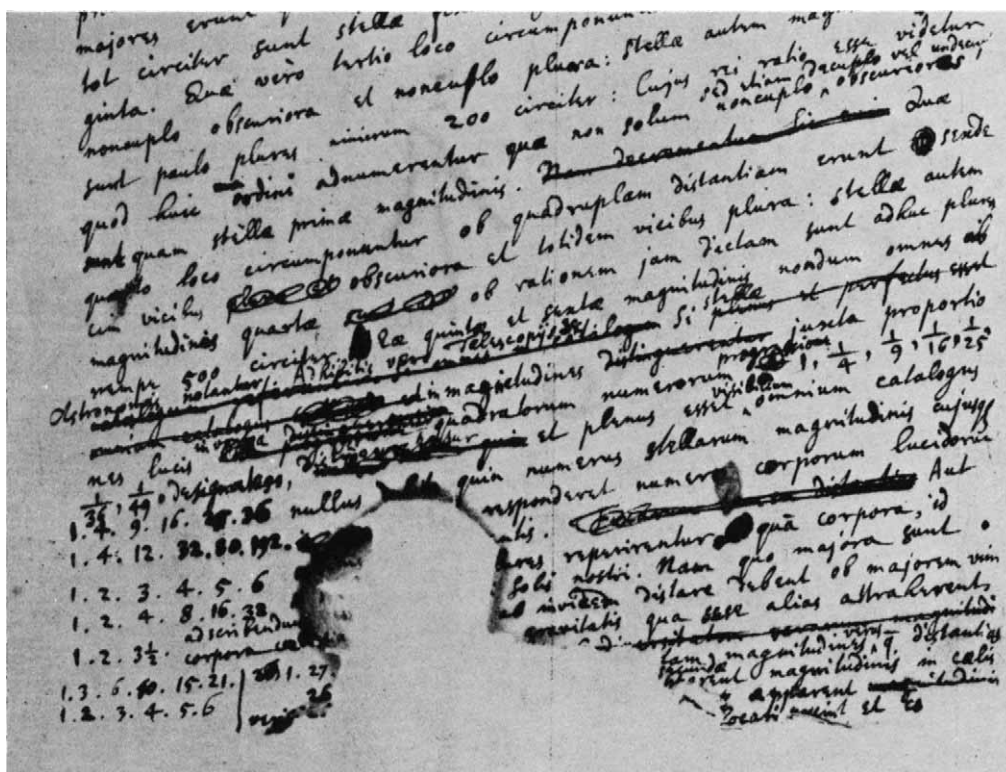


Fig. 6 Manuscript of Newton's cosmological work: f.74 with part of the calculation burnt away. (Permission of the Cambridge University Library.)

away. Since Sirius is somewhat brighter than the first magnitude, it should be closer; it actually lies at about 550,000 astronomical units, so we can see that in Newton's day the distances to the nearer stars were surprisingly well understood.

Intimately related to the concept of photometric distances is the puzzle that has come to be known as Olbers's paradox. If the stars are set out in an infinite and regular system, the more distant stars should exactly compensate for their diminishing brightnesses by their greater numbers. Such calculations lead us to expect that the whole sky should be ablaze with light of the intensity of the sun. Olbers, and de Chéseaux who worked on the problem before him, concluded that imperfect transmission of light in space might provide the answer; today, both the expansion of the Universe and the finite lifetime of stars are credited with producing a dark sky.

A closely comparable problem occurs in the case of gravitation, which, like light, diminishes according to the inverse square of the distance. In the early 1690s Newton was forced to think about this question when Richard Bentley asked him why the system of stars did not collapse into its own centre under the influence of gravity. Newton essentially side-stepped this cosmological dilemma in later editions of his *Principia*, and until a few years ago no one realised that Newton had wrestled with this issue. In 1970, suspecting that Newton may have considered the problem, the Cambridge historian of science Michael Hoskin⁸ searched through the boxes of Newton manuscripts in the Cambridge University Library. Several days of detective work resulted in the recovery of drafts of a new proposition that Newton had once planned to include in the second edition of the *Principia*.

In effect, Newton reasoned that in an infinite universe the forces would just balance out and keep the stars from falling together. To convince himself that this would work, Newton was obliged to show that the distribution of stars in space was uniform, so that the greater number of more distant stars would exactly counterbalance their diminishing gravitational

attraction (just as for the distribution of brightness in Olbers's paradox). Hoskin found the very calculations with which Newton tried to sort the distances of stars according to their apparent magnitudes. Newton must have been within a hair's breadth of the paradox, but he failed to draw what is to us an obvious conclusion. Hoskin has written: "Interestingly, the paradox was propounded in public, for—as far as we know—the first time, by Edmund Halley in 1721, at a meeting of the Royal Society. Was Newton present? It so happens we can answer that question, because as President of the Royal Society he took the chair at the meeting. We have no reason to think the paradox worried him. Perhaps he was satisfied with the confused and erroneous refutation which Halley offered—or perhaps he was quietly sleeping."

In retrospect, the astronomy of three hundred years ago was a lively, inquiring enterprise. The appointment of Flamsteed as the "Royal Observer" and the foundation of the Greenwich Observatory in 1675 were characteristic of the vitality that brought the world focus in astronomy to England. Isaac Newton was of course the dominating figure; his astronomical insights ranged from the figure of the Earth to the infinite Universe. In spite of the personality clash with Flamsteed, Newton's interaction with the Astronomer Royal and the Greenwich Observatory led to important achievements in Newtonian astronomy—the achievements that set the stage for the ensuing century, and which still mould our science today.

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articles

Ice ages and the Galaxy

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The passage of the Solar System through a dust lane bordering a spiral arm of the Galaxy may cause a temporary variation of the Sun's radiation and so lead to an ice epoch on Earth.

RECENT contributions to *Nature*^{1,2} indicate that there still exists no consensus as to what agency triggers the onset of an ice age on Earth. Most discussions make passing mention of some possible astronomical agency, but without any critical up-to-date examination. The purpose of this paper is to show that one particular astronomical hypothesis gains much in plausibility in the light of recent far-reaching advances in our knowledge of the entire Milky Way Galaxy—and, indeed, of the behaviour of galaxies in general. Certain processes which we could previously say might occur, we can now say almost certainly must occur and must do so in ways that seem to account for several characteristic features of ice ages.

In a remarkable paper³ published in 1939 on "The effect of interstellar matter on climatic variation", Hoyle and Lyttleton suggested that the passage of the Solar System through a cloud of interstellar matter could result in an ice age on Earth. Sir George Simpson had inferred that a slight increase in the flux of solar radiation reaching the Earth is needed to initiate an ice age by leading to an increase in the rate of precipitation. Hoyle and Lyttleton showed that matter might fall into the Sun from an interstellar cloud at a rate such that the release of gravitational energy would produce a temporary brightening of the Sun by the requisite amount. They were able to infer that the hypothesis would account qualitatively for several associated properties. Their work has been quoted repeatedly since, although conclusive comments have been lacking. On the geological and meteorological side, the inconclusiveness has been associated with the absence of positive predictions; the work had shown rather what could occur than what had to occur. On the astronomical side, it required cloud densities much greater than, until very recently, astronomers deemed credible; also the general astronomical significance of accretion came to be largely discredited and unfortunately this particular application suffered unduly in consequence.

Energy-release in accretion

Hoyle and Lyttleton inaugurated extensive studies of the accretion process in many astronomical contexts. For the present purpose the simple theory in their paper will still suffice; using so far as possible the original notation, it is:

Let the Sun of mass M and radius R move with uniform velocity v through a cloud having uniform (undisturbed) density ρ . The gravitational attraction of the Sun deflects material into the wake of the Sun where its transverse motion is destroyed by mutual collisions. Out to a certain distance λ , say, such material is left with less than escape speed. It will fall into the Sun, thereby releasing energy GM/R per unit mass, where G is the gravitational constant, since it falls from

distances large compared with R . Simple dynamics shows that in this way the Sun sweeps up all the cloud material within distance σ of its path, where

$$\sigma = 2 GM/v^2 \quad (1)$$

and in fact $\lambda = \sigma$. The rate of accretion is therefore $dM/dt = \pi \sigma^2 \rho v$. Thus the resulting rate of energy release is a fraction ϵ of the normal luminosity of the Sun L , where

$$\epsilon = \frac{GM}{RL} \frac{dM}{dt} = 4\pi \frac{G^3 M^3 \rho}{RLv^3} \quad (2)$$

If the parameters are initially in cgs units, it happens to be convenient to write $\rho = nH_2$, where H_2 is the mass of the hydrogen molecule, and n is the density in equivalent hydrogen molecules per cubic centimetre, and also to write $v = 10^6 V$, where V is then the speed in km s⁻¹. Figure 1 is a logarithmic plot of n against V for $\epsilon = 1, 10, 100\%$, using solar values of M, R, L . It also shows σ in astronomical units plotted against V .

This simple theory may be criticised for treating the cloud-material as composed of independent particles rather than as a compressible fluid. But later elaborations of accretion theory^{4,5} tend to show that the simple theory should give valid orders of magnitude. As regards the energy released, all that signifies is that the computed mass finds its way on to the Sun from distances much greater than the solar radius. Then the stated amount of gravitational energy must be liberated; the detailed mechanism need not concern us here. Hoyle and Lyttleton showed also that ancillary effects like the gravitational attraction of the cloud as a whole need not be considered in this connection. The resistance of the cloud⁶ is probably unimportant in cases of interest here but any effect it has would be favourable to the processes under discussion.

Astronomical relevancies

What is seen as a spiral arm of a galaxy like our own is a region where there are (short lived) very bright stars illuminating clouds of interstellar gas and dust. Stars and gas must move through the arms; otherwise, because of the observed differential rotation, the arms would have become tightly coiled. Observation thus shows that the spiral structure is some wave-pattern moving through the disk of stars and gas; or, what is the same thing, it is a pattern through which these objects move. Observation leads to the further inference that these objects enter an arm at its inner (concave) edge, except perhaps in the outermost parts of the system. Furthermore, observation often shows a dust lane along this edge. This is interpreted as a shock-wave effect whereby the clouds become temporarily compressed as they cross this lane. It may be inferred that the compressed clouds are the sites of formation of clusters of new stars, the most massive of which are the very bright stars that illuminate the arm as they proceed on their way but burn themselves out before emerging into the interarm

region. At each passage through an arm, only a small fraction of the total diffuse matter can be formed into stars; otherwise very little could still remain. All this interpretation is supported by many other observations; it depends on no particular theory of spiral arms although, as is well-known, a density-wave theory has been elaborated.

On this picture, the Sun and its planetary system were formed in a compressed cloud some 5×10^8 yr ago; since then it has circulated round the Galaxy some 20 times. It can be estimated that it crosses a spiral arm at intervals of the order of 10^8 yr, spending the order of 10^7 yr crossing the main part of the arm and the order of 10^6 yr crossing the compression lane. So it has passed through an arm some 50 times in all. At present, astronomers plot its position as just inside, or just about to enter, the inner edge of the so-called 'Orion arm'; consequently we infer that it entered the associated compression lane a million or more years ago and left it only comparatively recently.

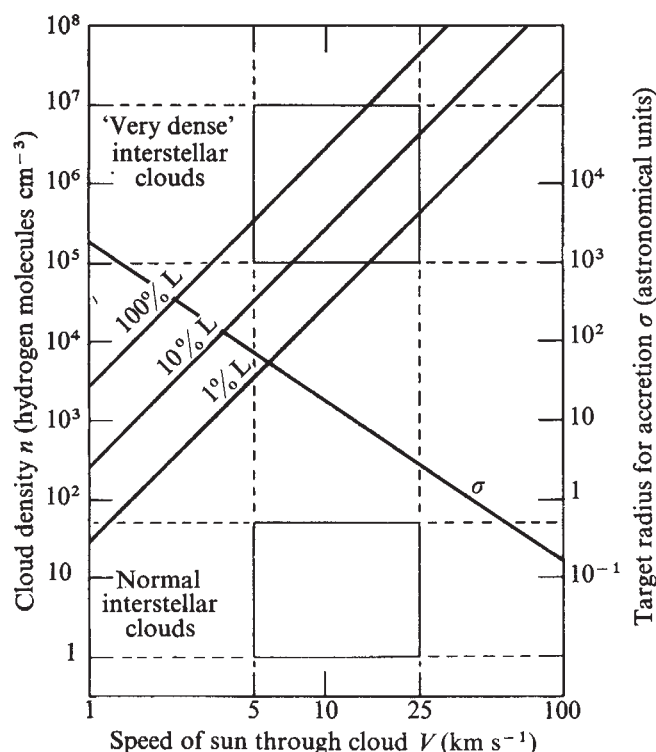


Fig. 1 If the Sun has velocity V through a cloud of density n , the material within distance σ from its path given by equation (1) falls into it, thereby releasing energy at rate ϵL given by equation (2), L being the Sun's luminosity.

Knowledge of interstellar clouds has also advanced greatly in recent years; the discovery and study of an unexpected variety of molecules therein has led to remarkable inferences about their densities in certain cases. In 1972 Lequeux⁷ reviewed some of the pioneering recent developments. In particular, he described three 'very dense' clouds: one with a mean density of about 10^5 hydrogen molecules per cm^3 over a distance of 10–15 pc, one for which he estimates about 4×10^5 molecules cm^{-3} over about 0.33 pc, and a third with a density "probably much larger than 10^7 cm^{-3} over an extent of at least 1.5 pc". Since the 'compression lanes' are apparently the densest extended regions of interstellar matter in a galaxy, and since densities in the range just quoted are actually inferred, we can only conclude that the densities in the lanes reach at least this range of values. For 'normal clouds' Lequeux quotes a density range which in the same units is 1–50 cm^{-3} . These ranges are indicated in Fig. 1.

Ice ages

According to a summary given by Allen⁸, ice epochs occur on Earth with a period of about 250 Myr, each epoch having a duration of a few million years and consisting of several glaciations with period (irregular) of glaciation and interglaciation about 0.25 Myr, the glaciation itself lasting about a fifth of the period. The most recent epoch was the Quaternary, which is inferred to have begun about a million years ago, and the latest glaciation ended about 11,000 yr ago. In the geological literature there seems to be a good deal of uncertainty about the whole subject; nevertheless it seems to be fairly generally agreed that there is a characteristic time of the order of 10^8 yr for the occurrence of an ice epoch, and a characteristic duration of the order 10^6 yr, each epoch consisting of several glaciations. ('Ice age' seems to be used sometimes for 'ice epoch' and sometimes for 'glaciation'; it seems best to speak of the whole set of phenomena as 'ice ages'.)

Implications of repeated dust lane encounters

Since the speed of the Sun relative to the nearer stars is about 20 km s^{-1} and since there is no known systematic difference between the motions of stars like the Sun and of interstellar material in the same region of the Galaxy, this must be also about the Sun's speed relative to the nearer interstellar clouds. Further, Allen⁸ quotes for the r.m.s. random velocity of the clouds in the line of sight a value 9 km s^{-1} . So the speed of the Sun V relative to an individual cloud which it encounters may be taken to be in about the range $5\text{--}25 \text{ km s}^{-1}$; this seems to be the sort of range usually considered in the literature in discussing related problems; it is indicated in Fig. 1. The combined ranges of V and n for 'normal' interstellar clouds are shown by the lower rectangle in the figure. We see that any accretion in such conditions can have only a negligible effect on the Sun's luminosity. Thus the passage of the Solar System through a normal interstellar cloud has no significance in the present content.

In Fig. 1 the combined range of V and n for 'very dense' clouds is shown by the upper rectangle. It is remarkable how this is at once seen to produce conditions of crucial interest. Throughout practically the whole range, accretion could produce a temporary brightening of the Sun by over 1%, and it may exceed 100% in part of the range. The elementary theory may somewhat overestimate the possibilities, nevertheless there is ample margin for asserting that something significant can and must occur.

Lequeux's figures suggest also that, if the Sun enters a dense cloud, it is likely to travel a distance of the order of a parsec through it. At, say, 20 km s^{-1} this would take about 50,000 yr.

For the proportion of space near the galactic plane occupied by clouds Allen⁸ quotes a figure of 4%. This implies that a star moving through such a region would spend 4% of its time inside some cloud. Were the same proportion to apply in a region of dense clouds, then the time just mentioned would imply an average interval of the order of 10^6 yr between immersions. But the appearance of the dark lanes suggests that clouds may be much closer together there; so a million years may be an overestimate for these regions.

If now we accept the general line of reasoning of Hoyle and Lyttleton about the effect of a temporary increase in the solar radiation and if we combine the present results with the foregoing inferences regarding the encounters of the Solar System with compressed clouds, we then infer: at intervals of the order of 10^8 yr—when the Solar System enters a compression lane—there must occur on Earth an ice epoch. A glaciation must last on average the order of 50,000 yr—the time of travel through a particular cloud. The Solar System would take a few million years to cross the lane, and several glaciations would be expected to occur within that time. Since the Solar System is near the inner edge of the Orion arm, it has presumably 'recently' crossed an associated lane; so

there should have been an ice epoch during the past few million years extending up to near the present time. When the Solar System is not in a region of very dense clouds, no such effects would occur.

All this agrees remarkably well with what is known about ice ages as summarised in the preceding section. On the one hand, there is naturally much more to be done on both the astronomical and the meteorological aspects. In particular, the way in which a change in the Sun's radiation might trigger an ice-age is still conjectural, and if one has been triggered it could be that some ebb and flow of ice could persist for quite a long time before that ice age could be said to be past. On the other hand, there are compulsive features; very dense clouds do exist and the Sun must go through them and when it does its radiation must be affected. If therefore this can be accepted as the explanation of ice ages, other features in the overall picture may be regarded as confirmed, such as the behaviour of the Sun relative to the Orion arm of the Galaxy. Of course, if ice ages are satisfactorily accounted for in this way, there is less support for an explanation⁹ depending on intrinsic variability of the Sun. Also it is now worth looking for other

effects that may follow from the causes discussed here, as I have attempted to do elsewhere (Halley Lecture, Oxford 1975).

Recently my attention has been called by Professor Kiang of Dunsink Observatory to the fact that the late Harlow Shapley^{10,11} long ago anticipated in principle the basic ideas of both Hoyle and Lyttleton and the present work. Shapley wrote at times when relevant astronomical knowledge was more tentative and restricted, but his insight into its possible significance was astonishing. I regret not knowing of his suggestions at an earlier stage.

Received April 8; accepted May 6, 1975.

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Structure of chicken muscle triose phosphate isomerase determined crystallographically at 2.5Å resolution

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using amino acid sequence data

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Each subunit of triose phosphate isomerase is composed of alternate segments of polypeptide chain in the α - and β -conformations that are arranged to form an inner cylinder of parallel-pleated sheet and a largely helical outer shell. Residues participating in the subunit interface and the active sites have been identified.

As a contribution to the study of glycolytic enzymes now making rapid progress in a number of laboratories¹ and to explore the accessibility of an isomerisation reaction to direct crystallographic study, we have investigated the three-dimensional structure of triose phosphate isomerase (TIM) by combined X-ray and chemical analysis. Triose phosphate isomerase (D-glyceraldehyde 3-phosphate ketol

isomerase: EC 5.3.1.1.) catalyses the interconversion of dihydroxyacetone phosphate (DHAP) and D-glyceraldehyde-3-phosphate (GAP), a crucial isomerisation in the Embden–Meyerhof–Parnas pathway of glycolysis. After preliminary crystallographic work on the rabbit muscle enzyme², which established that the molecule of overall molecular weight about 52,000 is a symmetrical dimer, our attention was directed to chicken-muscle TIM through the observation of Scopes³ that this material is relatively free from isoenzymes, showing only one minor contaminant with a single major activity. Preliminary experiments in Bristol by one of us (C.I.P.) showed that it crystallises readily from 10 mg ml⁻¹ solution in 0.1 M triethanolamine hydrochloride 5 mM EDTA, 5 mM mercaptoethanol, pH 7.4 at 20 °C by the addition of solid ammonium sulphate to 60% saturation. The crystals are orthorhombic⁴ with space group P2₁2₁2₁ and cell dimensions $a=106.01\pm0.19$, $b=74.76\pm0.12$, $c=61.74\pm0.06$ Å (refs 5 and 6). There is one dimeric molecule per asymmetric unit comprising two chemically identical polypeptide chains each of 247 amino acid residues.

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Table 1 Amino-acid sequence of chicken muscle triose phosphate isomerase

A	P	-	R	K	F	F	V	G	G	N	W	K	M	N	G	K	R	K	S	10	20
L	G	E	L	I	H	T	L	D	G	A	K	L	S	A	D	T	E	V	V	30	40
C	G	A	P	S	I	Y	L	D	F	A	R	Q	K	L	D	A	K	I	G	50	60
V	A	A	Q	N	C	Y	K	V	P	K	G*	A*	F*	T*	G	E	I	S	P	70	80
A	M	I	K	D	I	G	A	A	W	V	I	L	G	H	S	E	R	R	H	90	100
V	F	G	E	S	D	E	L	I	G	Q	K	V	A	H	A	L	A	E	G	110	120
L	G	V	I	A	C	I	G	E	K	L	D	E	R	E	A	G	I	T	E	130	140
K	V	V	F	Q	E	T	K	A	I	A	D	N	V	K	D	W	S	K	V	150	160
V	L	A	Y	E	P	V	W	A	I	G	T	G	K	T	A	I	P	Q	Q	170	180
A	Q	E	V	H	E	K	L	R	G	W	L	K	T	H	V	S	D	A	V	190	200
A	V	Q	S	R	I	I	Y	G	G	S	V	T	G	G	A	C	K	E	L	210	220
A	S	Q	H	D	V	D	G	F	L	V	G	G	A	S	L	K	P	E	F	230	240
V	D	I	I	N	A	K	H														

Residues forming part of the supposed active site are shown in circles and those playing a part in the inter-subunit contact are enclosed in squares. Residues forming part of the supposed active site of the adjacent subunit are shown in circles with an asterisk. Residues forming helices are underlined with a wavy line (α -helices) or a chain symbol (3_{10} -helices) and those forming β structure have a line of circumflexes above them. For the one-letter code for amino acids see ref. 33.

These crystals have been the subject of the present study.

Chemical studies were begun in close association with the crystallography and the complete amino acid sequence of rabbit muscle TIM has been determined by Corran and Waley⁷ even though first priority for crystallographic studies was transferred to the chicken muscle enzyme when the crystals of this material proved to be more suitable for X-ray analysis. Analysis of the chemical sequence of chicken TIM was planned as a collaborative effort with the crystallography in the hope that a good electron-density map would provide the information needed to place the tryptic peptides in order. In the event, however, it was possible to do this by consideration of the fragments produced by cyanogen bromide digestion and comparison with the rabbit TIM sequence before the final electron-density map was

calculated. Furth *et al.*⁸ have reported a sequence for chicken TIM based on this evidence (Table 1).

An account of preliminary crystallographic studies at 6 Å resolution has been published earlier⁹ and we report now the complete determination of the structure of chicken muscle TIM by the interpretation of an electron-density map at 2.5 Å resolution in terms of the chemical data.

Crystallography

Five isomorphous derivatives were used in the X-ray analysis of the structure by the method of multiple isomorphous replacement supplemented by the use of anomalous scattering data. Three of them were prepared with mercurial compounds of different kinds all of which react with the most reactive sulphhydryl group in each subunit (now seen to be

Table 2 Heavy atom parameters used in phase determination

	Site no.	X	Y	Z	Occ. (electrons)	B (Å ²)	R(F _{HLE})
MNP	1	0.1253	0.0091	0.7312	87.7	6.3	0.523
	2	-0.0052	0.2403	0.1595	73.4	18.3	
	3	-0.0042	0.2445	0.0863	23.2	1.3	
	4	-0.0192	0.2872	0.1124	20.1	18.8	
EMP	1	0.1237	0.0104	0.7296	87.2	26.1	0.510
	2	-0.0072	0.2421	0.1620	60.3	2.8	
	3	0.0032	0.0493	0.0553	43.8	22.1	
	4	0.0605	0.1924	0.6729	34.8	5.7	
BK	1	0.1253	0.0130	0.7203	16.3	-19.0	0.501
	1A	0.1225	0.0090	0.7685	43.0	-1.3	
	2	-0.0026	0.2500	0.1643	18.1	-1.4	
	2A	0.0087	0.2432	0.1927	24.5	13.0	
	3	0.0802	-0.0262	0.7748	42.0	34.8	
	4	0.0463	0.2659	0.1537	27.4	34.0	
PT24 (6-3 Å)	1	0.1193	0.0061	0.0090	11.8	4.0	0.559
	2	0.4526	0.2767	0.2136	40.1	-2.9	
PT24 (3-2.5 Å)	1	0.1137	0.0297	-0.0038	12.2	22.6	0.558
	2	0.4485	0.2775	0.2218	54.3	0.0	
PT27 (6-3 Å)	1	0.1237	0.0111	0.0145	49.1	42.0	0.557
	2	0.4521	0.2733	0.2214	73.4	3.3	

$$R(F_{\text{HLE}}) = \frac{\sum |F_{\text{HLE}} - F_{\text{HLE}}^{\text{calc}}|}{\sum F_{\text{HLE}}}$$

For derivative abbreviations see text.

Cys 217) and consequently have two sites per molecule in common. The first derivative was prepared in Bristol by co-crystallisation of the enzyme with 2-chloromercuri-4-nitrophenol (MNP, see Table 2)¹⁰ which binds mainly at these sites. The other derivatives were prepared by diffusion of reagents into existing crystals. Baker's dimercurial (BK)¹¹

provided additional sites close to the primary ones whereas ethyl mercury phosphate (EMP), presumably because of its smaller size and more hydrophobic character, reacted also with a second and less readily accessible pair of sulphhydryl groups (Cys 41).

The remaining two derivatives were prepared, after some

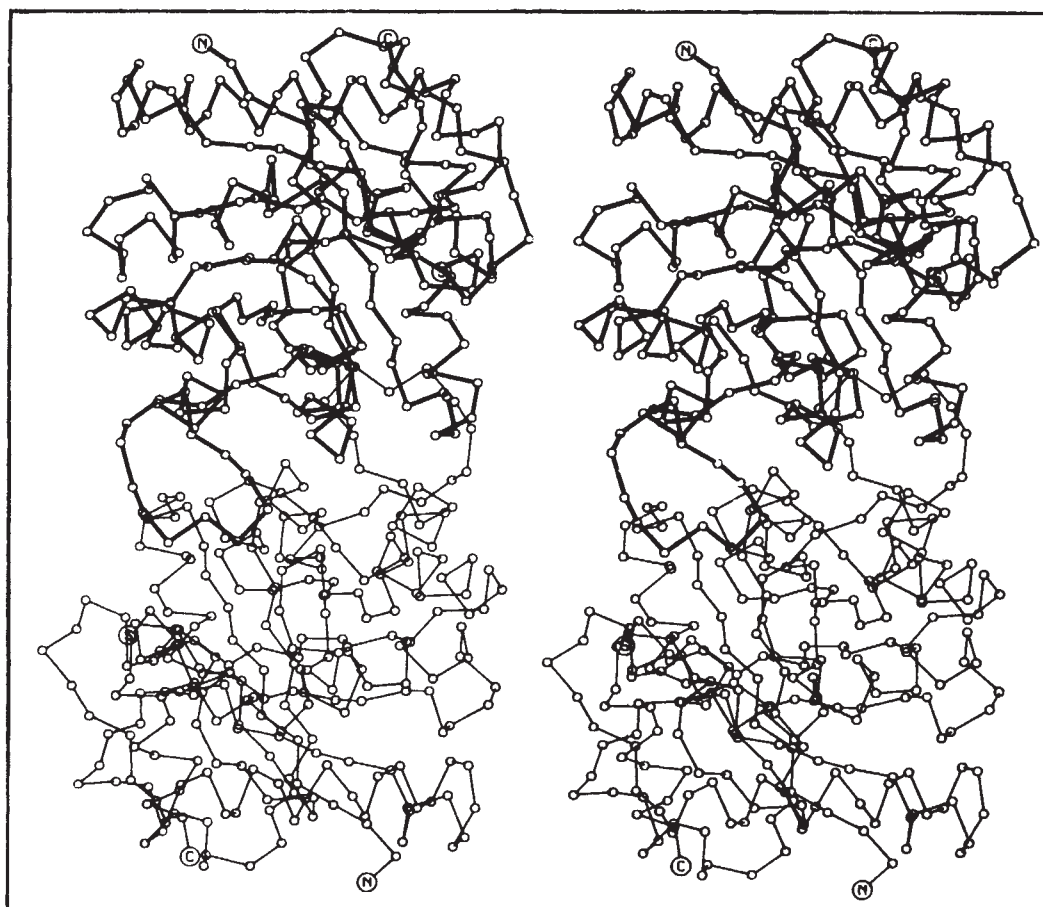


Fig. 1 Stereo drawing of the course of the main polypeptide chains in the dimeric molecule viewed down the non-crystallographic twofold axis. One subunit is shown in thicker line than the other. The amino- and carboxyl-ends of the chains are marked N and C and the position of the bound sulphate ion in the probable active site is marked S.

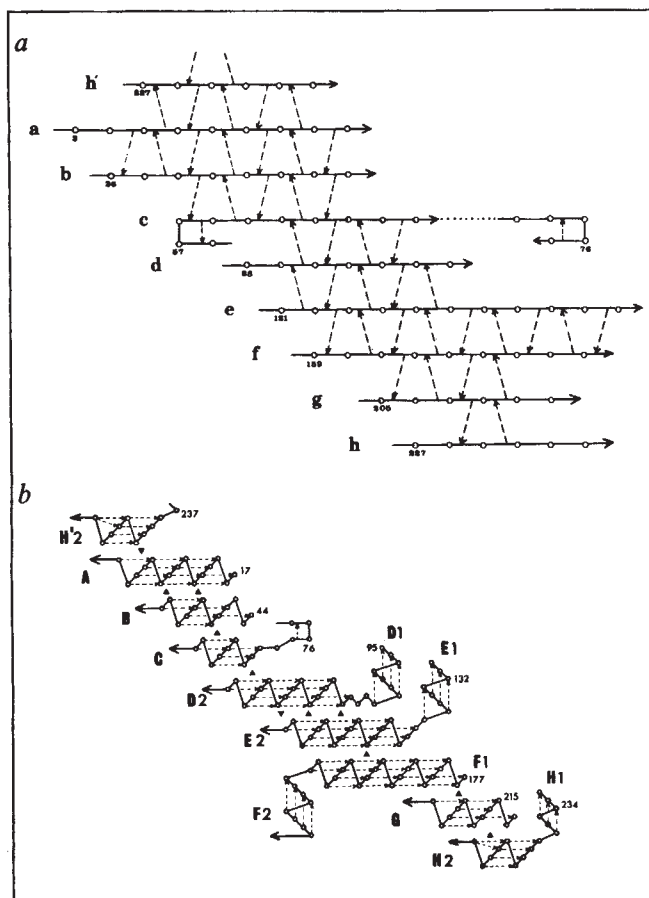


Fig. 2 *a*, Parallel β -pleated sheet structure in each subunit of TIM. Individual strands are labelled *a*, *b*, ... *h* with *h* repeated alongside *a* to emphasise that the sheet forms a closed cylindrical surface crosslinked by the hydrogen bonds indicated by broken arrows. Larger arrow heads point towards the carboxyl end of the polypeptide chain. *b*, Helices in each subunit of TIM. Helices occurring in the sequence between consecutive strands of β -sheet are denoted by capital letters (*A*, *B*, ... *H*) with individual helices, where necessary distinguished by numbers (*E*₁, *E*₂). Hydrogen bonds within helices are indicated by broken arrows. The arrangement of helices indicates roughly the contacts between them in the structure, with principal interactions denoted by closed triangles. They form a roughly cylindrical outer surface to each subunit in which the helices are generally anti-parallel to the strands of β -sheet.

difficulty, with $K_2PtCl_4(PT)$. Initially promising results with this compound^{5,6} proved difficult to reproduce, probably because the reactive ionic species is modified by interaction with the ammonia of the medium in a way that is not easily controlled¹². Following the suggestion of Sigler and Blow¹² it is now common practice to avoid this problem by transferring crystals to a phosphate medium but this course at first seemed to be unavailable because K_2PtCl_4 derivatives in phosphate had been observed to be non-isomorphous with the native crystals in either phosphate or ammonium sulphate. The difficulty was eventually overcome in two ways¹³, first by soaking a crystal (PT24) in 1 mM K_2PtCl_4 in 4 M mixed K_2HPO_4/NaH_2PO_4 for 48 h and then back-soaking it in 3.1 M ammonium sulphate for 1 h. Shortly after this success further experiments showed that crystals in 3 M mixed phosphate (rather than concentrations higher than about 3.3 M) would react with K_2PtCl_4 without losing isomorphism and the final derivative (PT27) was prepared in this way. The heavy atom is bound near His 26 and Lys 54. The preparation of these derivatives and the chemistry of K_2PtCl_4 interaction with proteins is described elsewhere (G.A.P., D.C.P., and R. J. P. Williams, unpublished).

Diffraction data for the native crystals and mercurial

derivatives were collected at about 20 °C by means of the linear diffractometer adapted to measure five reflections quasi-simultaneously¹⁴. The K_2PtCl_4 derivatives were measured near 0 °C, to minimise irradiation damage of the unique specimens¹⁵, by means of a five-circle diffractometer designed to measure five reflections quasi-simultaneously (D.W.B., P. R. Evans, D.J.M., and D.C.P., unpublished). Additional crystals and data were used to 6 Å resolution. After the usual processing the native and derivative data were scaled together over small volumes of reciprocal space¹⁶ to eliminate deficiencies in the corrections for absorption and irradiation damage.

The heavy-atom positions were found from difference Patterson maps and the parameters listed in Table 2 were refined by least squares adjustment of the heavy-atom structure amplitudes (F_H) to the experimental estimates of these quantities (F_{HLE}) derived independently for each derivative from the isomorphous and anomalous-scattering data^{17,18}. This method was found most satisfactory particularly for the sites common to the three mercury derivatives near the semi-special position $(\frac{1}{2}, 0, \frac{1}{2})$ and $(0, \frac{1}{2}, \frac{1}{2})$.

The phases of 17,125 X-ray reflections were calculated by the standard method^{19,20} and had an overall figure-of-merit of 0.73, falling to 0.56 at the 2.5 Å limit. The electron-density map was calculated on *z*-sections with spacing *c*/80. It was plotted to the scale $2 \text{ cm} \equiv 1 \text{ Å}$ with contours at intervals of 0.2 eÅ^{-3} (on an approximately absolute scale) starting from the r.m.s. error level of 0.2 eÅ^{-3} above the overall average density. The map sections were suspended for interpretation in a Richards' box²¹ with the mirror vertical and parallel to them.

Interpretation of the electron-density map

Although interpretation of the electron-density map in complete detail was made possible by knowledge of the amino acid sequence it may be noted that some 25% of the 494 residues within the dimeric molecule could be identified with some certainty from the map alone. As usual these include particularly the aromatic residues. The sulphur densities in the cysteinyl residues are rather low, however, and the densities at the β -carbon positions are often weaker than at other positions in either the main or side chains. In the whole molecule, 23 side chains are a poor fit with the map in regions where the electron density is relatively strong and these include six pairs of equivalent residues from the two subunits; 61 side chains lie in regions of weak density and these include five pairs from the two subunits. Only one short section of polypeptide chain, residues 168–176 in one subunit, could not be followed easily in the electron density. These facts illustrate the advantage of having two independent images of essentially identical structures to consider.

One amino acid residue in the published sequence⁸ is clearly not consistent with the electron density in either subunit. There is no room for an additional lysyl residue following Lys 19 and, although the residues that are present in this region cannot be identified with certainty from the map, it is likely that the sequence is that shown in Table 1 (in which the numbering has been adjusted to be consistent with the additional residue at position 3 in the rabbit sequence⁷).

The supposition that there is a lysine following Lys 19 was based upon the finding of a Lys–Lys peptide in the tryptic digest which cannot be fitted in elsewhere in the sequence⁸. Before confirmation of the whole sequence in the present study it was assigned to the position under discussion because this produces a local homology with the rabbit sequence. Examination of the electron-density map suggests no other likely place in which the extra lysine might be accommodated and the possibility thus exists that the observed dipeptide is the result of transpeptidation, a process

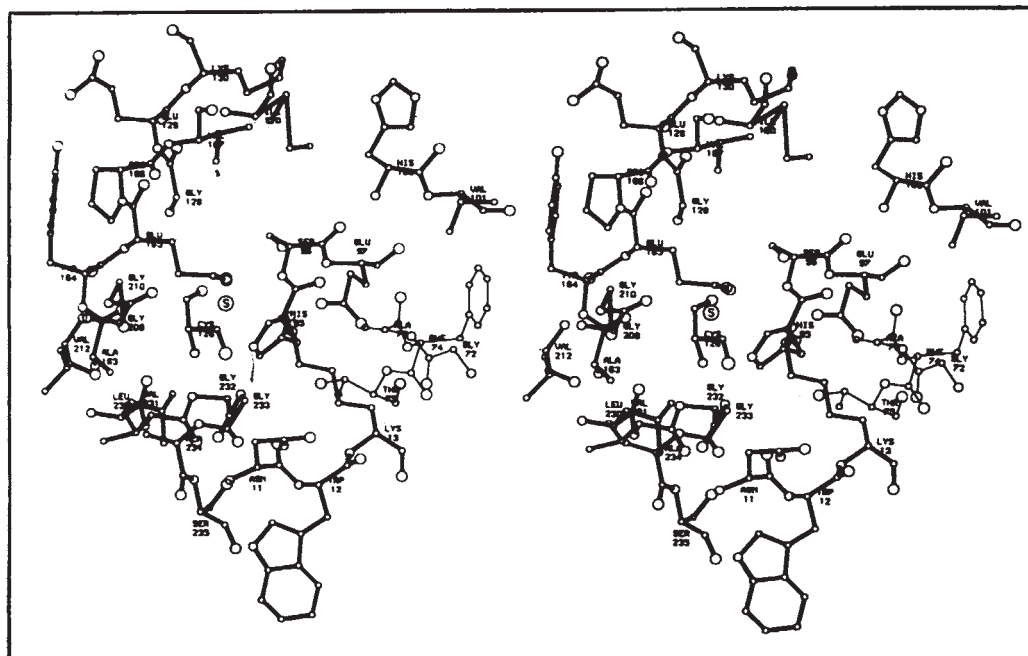


Fig. 3 Probable active-site region of one subunit. Amino acid residues within about 12 Å of the sulphate position are shown.

that is known to occur when peptides containing sequences of consecutive basic amino acids are incubated with trypsin²².

Description of the molecule

The course of the main polypeptide chain is shown in Fig. 1. Each subunit is roughly spherical with a diameter ~35 Å. Within each subunit the polypeptide chain is folded in a surprisingly regular manner to form an inner cylinder or barrel in which the staves are represented by eight strands of parallel β -pleated sheet. In keeping with the usual twist of β -pleated sheets²³ these strands are inclined to the axis of the barrel. Adjacent strands follow one another consecutively in the sequence and are linked by mainly helical segments of chain which form the outer surface of each subunit. To a first approximation this folding can be described as (sheet-helix)₈ or ($\beta\alpha$)₈, although the segments of regular secondary structure are not all the same and, in particular, the outer segments represented in this shorthand

simply by α often include more than one helix together with extended chain and, in one instance, an open loop (residues 70–80) which forms an important part of the inter-subunit contact.

This secondary structure is shown in more detail in Fig. 2 and it is indicated also in relation to the sequence in Table 1. Some 55% of the residues are found in helices and 22% in β -structures, almost entirely parallel-pleated sheets. The relationship between sequence and secondary structure is broadly consistent with the rules proposed by Chou and Fasman²⁴ and will be discussed elsewhere with reference to the sequences of chicken, rabbit, coelocanth²⁵ and *B. stearrowthermophilus*²⁶ TIM.

The general distribution of amino acid side chains is similar to that observed in other globular protein molecules with the groups shielded from contact with the surrounding liquid being non-polar or provided with complementary groups for hydrogen bonding. The subunit interface requires special mention. Apart from minor variations, mainly in

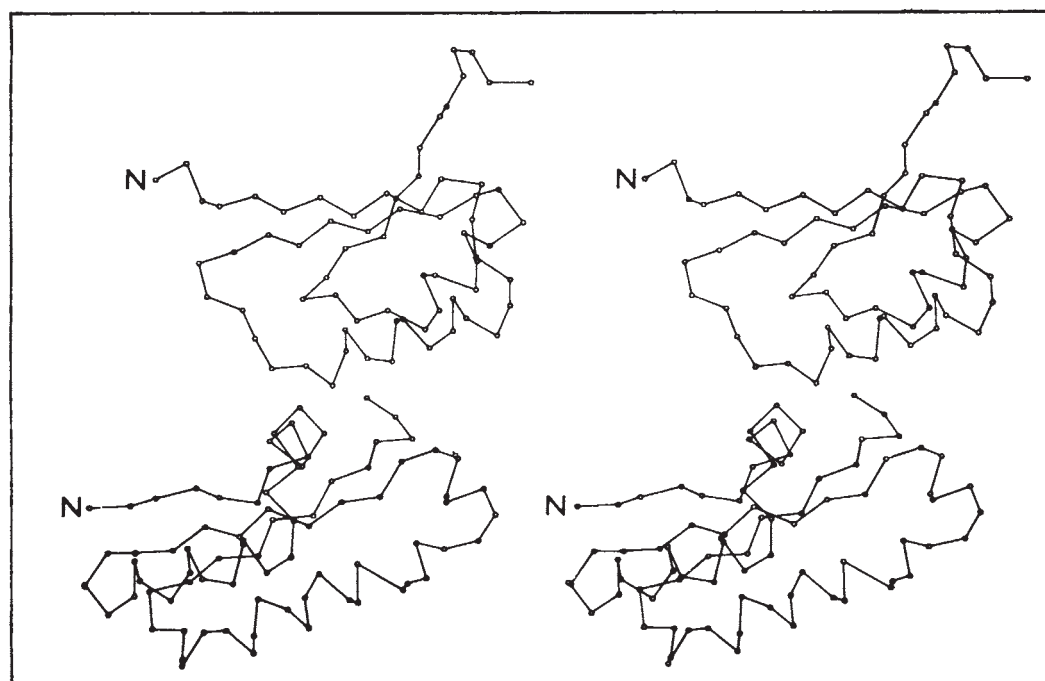


Fig. 4 "Super-secondary" structures formed by residues 1–70 (aAbBc) and 88–169 (dDeEf). The amino ends are marked N.

widely separated regions on the surface of the molecule, the two subunits are well related by a non-crystallographic twofold axis (in a direction perpendicular to Fig. 1). Their isologous interaction involves, in part, the loops 70–80 which form hydrophobic pockets around the residues Met 14 from the adjacent subunits. These pockets lie on the edges of the interface (Fig. 1), and the region between them, which is closer to the twofold axis, is more polar in character and includes a number of water molecules and ions that are apparently well ordered in the crystal. The residues principally concerned in the inter-subunit contact are indicated in Table 1.

The active sites

An important incentive for this study was the prospect of being able to observe directly a true enzyme–substrate complex with this enzyme⁹. Accordingly we have also examined crystals of the complex with dihydroxyacetone phosphate (DHAP) which, in 4 M phosphate, are not isomorphous with the crystals of the native enzyme⁴. The results of this study¹³ indicate that DHAP is bound to the enzyme at each subunit in the pocket shown in Fig. 3 and containing, among other residues of interest, the Glu 165 side chain that has been labelled by active-site-directed inhibitors^{27–29}. This possible active site lies at the carboxyl end of the β -barrel and is formed by residues (see also Table 1) from β -strands a, e, f, g, h, and helices D₁, E₁, H₁, together with a few, which include residues 72–75 from the adjacent subunit, in less regular conformations. In the electron-density map of the native enzyme this pocket includes indications of ordered water molecules and an ion, presumably sulphate, which may be bound near the position occupied by the phosphate group of a substrate (Figs 1 and 3).

Crystals of the enzyme–DHAP complex isomorphous with the native are obtained in 3 M phosphate¹³ and studies now in progress should reveal more clearly the position, conformation and orientation of the DHAP molecule bound to the enzyme and the extent to which the conformation of the enzyme changes when substrate is bound. The above details are presented since they suggest chemical experiments of the kind that will be needed to establish the nature and role of the functional groups participating in enzyme action. Thus, it may be relatively easy to modify Lys 13 by semi-synthetic methods³⁰.

Implications

At this stage in the analysis the most striking result is that triose phosphate isomerase resembles other glycolytic enzymes in being composed largely of alternating segments

of α - and β -structure that are often folded on each other in similar ways. Because of the cyclic nature of its ($\beta\alpha$)-structure, eight different examples of the $\beta\alpha\beta\alpha\beta$ -fold, first observed in subtilisin, flavodoxin and lactate dehydrogenase³¹, may be chosen in TIM (aAbBc, bBcCd . . . hHaAb in an obvious notation taken from Fig. 2) and each is generally similar to those found in the dehydrogenases and elsewhere³². Two are shown in Fig. 4. Furthermore it may be noted that the active sites of all the molecules with extensive parallel β structures that have been analysed in detail so far, are at the carboxyl ends of the pleated sheets, as we observe here in TIM. These findings must be taken into account in the discussion of the evolutionary significance of such structures now in progress, and they have redirected our attention to the suggestion by Rao and Rossmann³¹ that similar super-secondary structures may be found in many protein molecules with widely different amino acid sequences as the result of convergent evolution.

We thank the Medical Research Council for support, the Enzyme Preparation Laboratory of the Oxford Enzyme Group (SRC) which provided crystals of TIM, and the MRC, the SRC and the Rhodes Trust for studentships supporting A.C.B. and I.A.W., D.W.B., and G.A.P. respectively.

Received April 2; accepted May 2, 1975.

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Control of compartment development by the engrailed gene in *Drosophila*

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Our results demonstrate that the normal function of the engrailed (en) gene is required for the maintenance of the straight boundary between cells of the anterior and posterior compartments in the wing of Drosophila. We suggest that the activity of the engrailed gene is restricted to the posterior compartment where it 'labels' cells so that they do not mix with anterior cells during growth.

It has recently been discovered that organs in insects are subdivided into precisely defined regions called compartments¹, each being made by all the surviving descendants of a small group of founder cells^{1,2}. This founder group generates a polyclone³ of cells; that is, all their descendants exclusively form the compartment under consideration and no other cells contribute to it. Cells of the founder group are related to each other by position but not by ancestry. Although the whole polyclone always constructs the entire

Chromosomes	II	III	II	III
Parents	<i>lt stw en/+; M(3) i⁵⁵/+ × cn en/SM5; mwh jv/mwh jv</i>			
F ₁ (1)	<i>cn en/+; M(3) i⁵⁵/mwh jv</i>			
(2)	<i>cn en/lt stw en; M(3) i⁵⁵/mwh jv</i>			
(3)	<i>cn en/lt stw en; +/mwh jv</i>			

Fig. 1 Cross used in experiment 1. Three classes of offspring shown were analysed for clones homozygous for *mwh* and *jv* (see text).

compartment, the contribution of each of its members varies from individual to individual. During growth a polyclone can become subdivided into two daughter polyclones; before the subdivision a cell can give rise to progeny in both daughter polyclones, whereas afterwards a cell's progeny falls entirely into only one of the two daughter polyclones¹.

At least some compartments seem to be in the control of a small number of genes ('selector' genes⁴). When one of these genes is mutated, an entire compartment may develop the pattern of cell types appropriate for another: for example, in *Drosophila* mutant for the *bithorax* gene the anterior part of the haltere segment is transformed into anterior wing⁵. The border of this transformation seems to coincide with the antero-posterior compartment boundary. This, and other observations, have led to the hypothesis that the compartments are units for the genetic control of development. The idea is that the combination of active selector genes within a polyclone determines the compartment it will construct. These ideas are discussed at length elsewhere^{3,4}.

The *Drosophila* wing disk is subdivided into two major polyclones by the first larval stage when the wing disk consists of some 10–50 cells^{6–8}. One polyclone forms the anterior part of the wing and most of the dorsal thorax, the other makes the posterior part of the wing and the remaining parts of the dorsal thorax. The boundary separating anterior and posterior compartments in the wing blade runs between veins III and IV and is remarkably straight over most of its length.

The *engrailed* gene is an obvious candidate as a selector gene involved in this compartmentation of the wing⁴, because in *en/en* flies the posterior region of the wing blade develops vein patterns and bristles normally only found in the anterior region⁹. The mutation has no effect on the anterior region of the wing itself. The transformation of the posterior region is incomplete so that the posterior wing seems to be a mosaic of anterior and posterior patterns.

We suggest that the proper development and maintenance of a border between two compartments may depend on the confronting cell types being different, possibly as a response to the activity in one cell type, but not in the other, of a single selector gene. Where the cell types in neighbouring compartments are the same or similar we expect the border to be non-existent or abnormal.

One test of this conjecture is to look at the line separating anterior from posterior compartments in *engrailed* (*en/en*) flies (experiment 1) where the cells on either side of it, instead of being different, as in wild-type flies, are now similar. We have found that we cannot define an antero-posterior demarcation line in *en/en* wings although it is perfectly normal in *en/+* wings. Experiment 2 was designed to determine whether cells making the boundary depend locally on the action of the *en*⁺ allele. We therefore marked clones of cells mutant for *engrailed* (*en/en*) in an otherwise wild type wing (*en/+*). We find that, whereas *engrailed* clones in the anterior part of the wing have no effect on the pattern and define the normal border, *engrailed* clones in the posterior part frequently cross the border and may extend a long way into anterior territory. A full account of this work is pending (P. A. L. and G.M., unpublished).

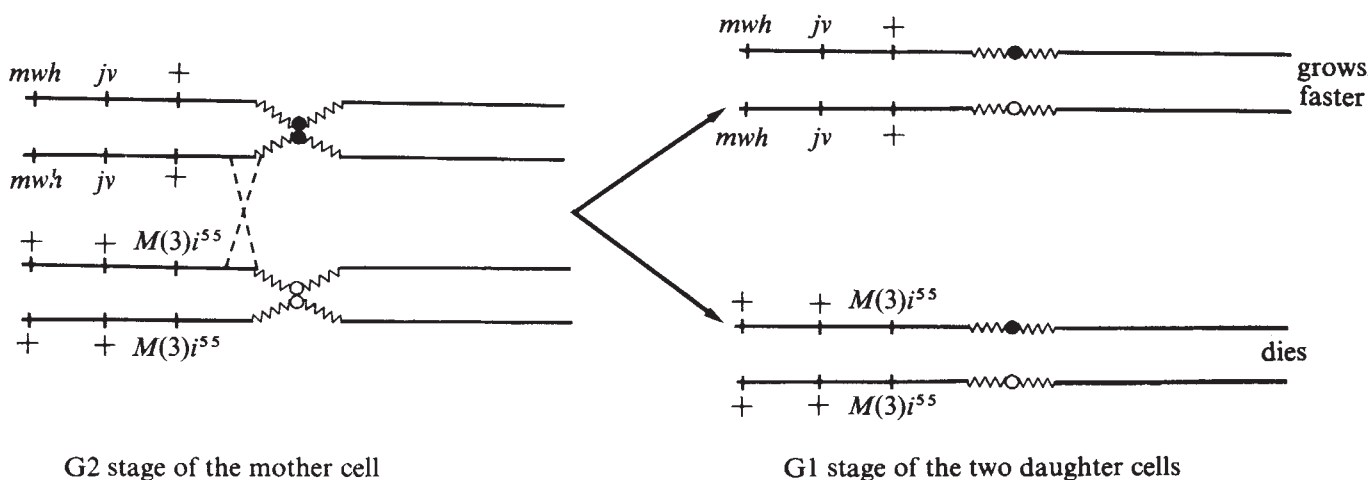
For experiments 1 and 2 we have used the *Minute* technique¹⁰: as a consequence of a single event induced by X rays, a cell may both become homozygous for a marker gene (which enables us to identify all its progeny) and gain the ability to grow much faster than all other cells in the disk. The clones these marked cells generate consequently give rise to as much as 90% of the compartment but are still unable to cross the compartment border. They therefore define the border for the investigator because their boundaries run along it for hundreds of cells.

The crosses for experiments 1 and 2 were set up as shown in Figs 1 and 3 and the results of somatic crossing over described in Figs 2 and 4. For the description of mutants used see Lindsley and Grell¹¹. F₁ larvae were irradiated with 1,000 rad (220 kV at 15 mA, 1 mm aluminium filter, distance 5 cm, rate 500 rad min⁻¹; rad=2.58×10⁻⁴ Ci kg⁻¹) at either 36±12 or 60±12 h after egg laying. Normally the insects were cultured at 25 °C, but in some experiments the bottles were transferred to 30 °C after the egg-laying period. The wings were mounted flat on slides for screening.

Experiment 1—marked clones in *engrailed* flies

We studied (Figs 1 and 2) one class of control flies which were *Minute* and wild type at the *engrailed* locus (*en/+; M(3)i⁵⁵/mwh jv*). After irradiation of these flies many *mwh*

Fig. 2 Third chromosomes of flies (classes 1 and 2, Fig. 1) to show how mitotic recombination produces daughter cells with different genotypes from the parental cell. Only crossing overs proximal to the *Minute* locus produce homozygous *M*⁺ clones which grow faster than the parental cell types.



Chromosomes	II	III	II	III
Parents	$M(2)c^{33a}/+; +/+ \times pwn\ en/SM5; mwh\ jv/mwh\ jv$			
F ₁	$pwn\ en/M(2)c^{33a}; mwh\ jv/+$			

Fig. 3 Cross used in experiment 2. F₁ offspring shown were analysed for clones homozygous for *pwn*.

clones (*mwh* cells secrete several wing hairs, *mwh*⁺ cells only one) will also be homozygous for $M(3)i^{25}$ and will therefore grow excessively. As expected from earlier work¹ very large *mwh* clones were found in these flies; many of them filled either the anterior compartment (Fig. 5a) or the posterior one (Fig. 5b). Out of the 128 clones which filled at least one-sixth of the compartment 69 ran along the antero-posterior boundary for at least half its length; none crossed it. Many of these clones extended on to both the dorsal and ventral wing surfaces.

We also screened two classes of *engrailed* flies. (i) The viability of flies of the genotype $en/en; M(3)i^{25}/mwh\ jv$ is very poor (less than 1% that of $en/+; M(3)i^{25}/mwh\ jv$) and few flies have been scored. There is also a consistent effect on the shape and size of the wing (also noted by R. B. Whittle, personal communication), the posterior compartment being much larger than normal. Of seven *mwh* clones none enabled us to define the antero-posterior boundary and three of them crossed the line where the boundary is in $en/+$ flies (Fig. 5c). (ii) Because we cannot take advantage of the M^+ effect in flies of genotype $en/en; mwh\ jv/+$, the clones were rather small and the boundaries much more difficult to define. Nevertheless 53 *mwh* clones were found, nine of which clearly crossed the line where the antero-posterior boundary is in $en/+$ flies, and several covered mutually overlapping territories.

These results show there is no straight line separating anterior and posterior compartments in en/en wings.

Experiment 2—marked *engrailed* clones in $en/+$ flies

For these experiments (Figs 3 and 4) we used the new cell marker mutant, *pawn* (*pwn*, 2-58)¹². *pwn/pwn* cells secrete truncated bristles and fine wing hairs with a basal spur. We also chose a *Minute* ($M(2)c^{33a}$) which is situated on the distal part of the right arm of chromosome II; flies heterozygous for this *Minute* emerge about 2 d later than controls¹³. The irradiated flies were screened for *pawn* clones and many large ones were found. These fell into two

distinct classes: (i) those clearly in the anterior region which frequently defined the boundary and had no effects on the bristles or veins; (ii) those in the posterior region, some of which reached the boundary, and if they did, frequently crossed into territory normally occupied by anterior cells. These clones, even if small, had effects on the venation, producing patches of veins and bristles characteristic of the *engrailed* phenotype. We conclude that the anterior clones can only have grown from cells within the anterior polyclone, whereas the posterior clones must have originated within the posterior polyclone. Anterior clones frequently filled the anterior compartment; they had no effect on the pattern of venation and defined the same antero-posterior demarcation line found in $en/+$ flies (Fig. 5d). Of 34 clones, 15 ran along the boundary for at least half of its length, none crossed it. This result shows that the *engrailed* mutation has no effect on any of the anterior cells, including those at the antero-posterior boundary.

Posterior *pawn* clones ($n=24$) always showed some aspects of the *engrailed* phenotype, and frequently crossed into anterior territory (11 out of 24). No clone defined the boundary and some extended a long way into the anterior part of the wing—even as far as the second vein (Fig. 5e). This result suggests that *en/en* cells in the posterior compartment express two kinds of anterior characteristics: they produce veins and bristles similar to those found in the anterior compartment; and they can become more miscible with anterior cells during growth, so that they can invade territory normally occupied by anterior cells.

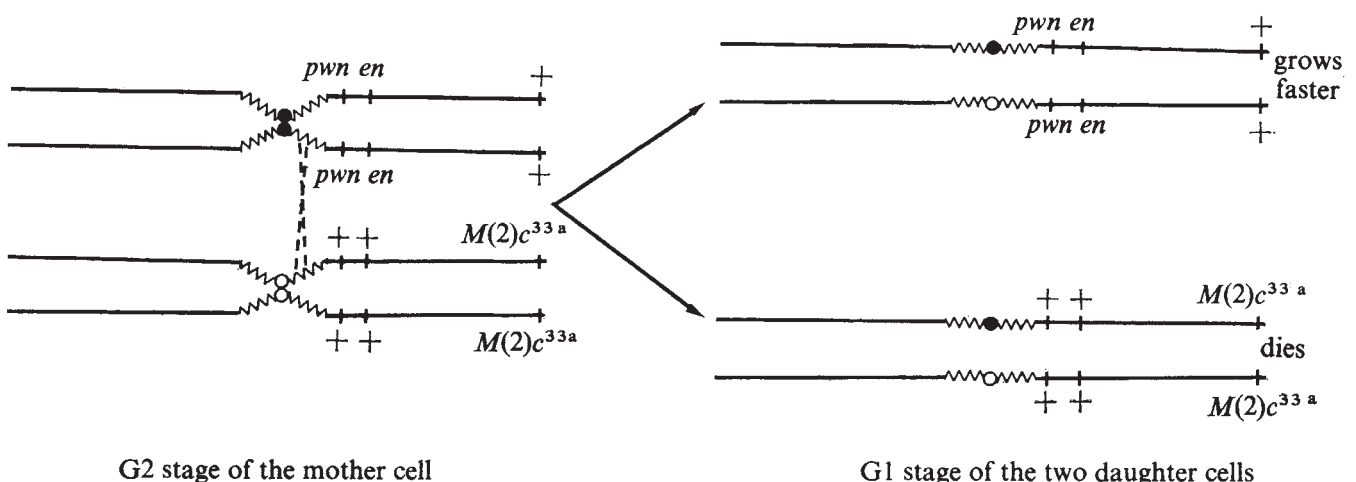
Both these characteristics are temperature sensitive because when experiment 1 was conducted at 30 °C (when the *engrailed* phenotype is very weak, the vein and bristle pattern being almost wild type) the clones respect the border almost perfectly.

In both experiments 1 and 2 clones homozygous for *engrailed* respected the dorso-ventral compartment boundary just as in $en/+$ files.

Discussion

A working hypothesis for the genetic control of compartment development can be outlined as follows. At the time of subdivision of a set of cells into two separate subsets (polyclones)³ a selector gene⁴ is activated in one polyclone and remains inactive in the other. After some growth a further subdivision involving another selector gene can occur in the two polyclones and produce two new compartments in each. Thus each compartment is genetically specified by the unique combination of active selector genes,

Fig. 4 Second chromosome to show the result of somatic crossing over between *pwn* and the centromere. All *pwn* clones are also homozygous for *en* and $M(2)c^{33a}$.



possibly depending on a binary code¹⁴. The product of each selector gene is continuously required in every cell so that the elimination of the wild type allele by somatic recombination results in a clone of cells which make a pattern appropriate to another (sister) compartment. The gene product of the selector gene is also involved, not necessarily directly, in producing some label on the cells so that during growth they do not intermingle with cells belonging to a neighbouring sister compartment, but instead form a straight and precisely positioned frontier with them. Elimination of the selector gene from cells in one compartment would remove the label so that the cells become miscible with cells of the sister compartment which always lack that label. Elimination of the selector gene in the sister compartment will be totally without effect.

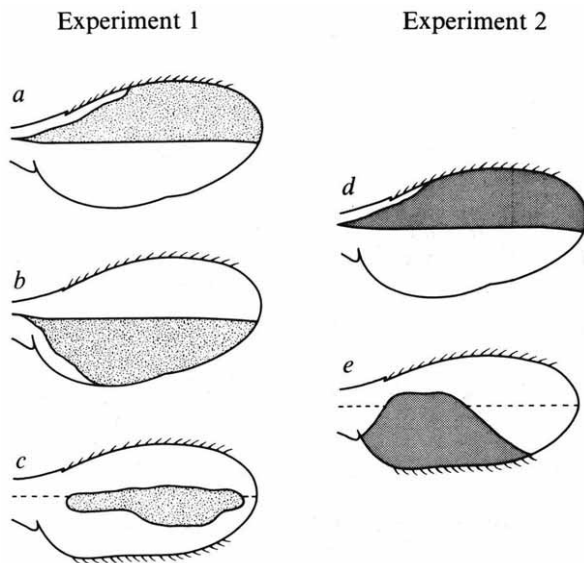


Fig. 5 Examples of clones produced in experiments 1 and 2 (diagrammatic). *a*, Anterior *mwh* clone in *en/+* wing; *b*, posterior *mwh* clone in *en/+* wing; *c*, *mwh* clone in *en/en* wing; *d*, anterior *pwn en* clone in *en/+* wing; *e*, posterior *pwn en* clone in *en/+* wing. *mwh* Territory, light shading; *pwn en* territory, darker shading; dotted line shows position of compartment border.

Our experiments with *engrailed* provide substantial support for this hypothesis; they show that the *en*⁺ allele is critical for the normal development of the posterior compartment and for the segregation of its cells from the anterior ones.

Our results—the incomplete transformation of posterior into anterior pattern, the temperature sensitivity—also suggests that *engrailed* is a leaky mutation. This conclusion explains our observations that posterior *engrailed* cells can mix during growth, both with anterior *en*⁺ cells (they cross the compartment border) and with posterior *en*⁺ cells (they fail to sort out from them). We would predict, however, that clones of cells in the posterior compartment, which completely lacked the *en*⁺ gene, would be perfect anterior cells and would mix with cells of the anterior compartment and sort out from those of the posterior. Nevertheless, our results show that when the cells of the posterior compartment are mutant for *engrailed* they cannot define the normal antero-posterior boundary either in an *engrailed* background (experiment 1) or in wild type background (experiment 2).

Experiment 2 also defines the realm of action of the *engrailed* gene: removal of the *en*⁺ allele from all the anterior cells is without effect, whereas its removal from the posterior cells produces changes in phenotype, and may

affect the compartment boundary. This strongly supports our hypothesis that the gene is active in all the cells of the posterior polyclone and inactive in the anterior polyclone. The dependence of the posterior compartment on the product of the *engrailed* gene is underlined by the effects of temperature: when the *engrailed* phenotype is nearly suppressed at 30 °C, the mutant posterior cells can now define an almost normal antero-posterior boundary. It is only the antero-posterior compartment boundary that is dependent on *engrailed*; the boundaries separating dorsal from ventral compartments, which are the next to appear on the wing¹, develop normally in *engrailed* flies.

The difference between wild type anterior and posterior cells has been studied *in vivo*. The two cell types (one genetically marked) sorted out and did not form mosaic patterns after being dissociated, mixed and reaggregated¹⁵. Similar experiments with *engrailed* wing disks demonstrated that at least some of the posterior cells show the same or similar affinities as the anterior cells⁹. Although we know little of the mechanisms subdividing one set of cells into two polyclones, it is possible that these specific cell affinities may function in keeping cells of the two nascent polyclones segregated. It therefore seems likely that the *engrailed* gene is involved from the earliest stages of anterior and posterior compartment formation.

Our experiments suggest that the *en*⁺ allele labels the posterior cells. Removal of this allele is followed by a partial loss of the label and this makes the posterior cells miscible with anterior cells. The label is continuously dependent on the activity of the *en*⁺ allele until late in development⁹, the activity of the gene being responsible for the boundary separating cells of the anterior and posterior compartments. The straightness of this compartment border could be caused by two cell populations maximising intercellular contact within each population while minimising the area of contact between them. A similar suggestion has been proposed to explain the straight line separating segmental compartments in *Oncopeltus*^{16,17}.

The *engrailed* gene is not only crucial to wing development but seems to be involved in a homologous way with the antero-posterior separation in other disks—possibly in the first pair of legs¹⁸ and the halteres and perhaps in all thoracic segments⁴.

The ability of cells to sort out from other cell types, and specifically aggregate with cells of the same type, has been studied in many other systems^{19,20}. There is the possibility that these properties may be serially acquired during development. Our view, arising from our experiments, is that their role *in vivo* may not be to sponsor aggregation, but rather to stop differently determined sets of cells from mixing.

We thank our colleagues in this laboratory and R. B. Whittle for discussion, S. M. Green and B. Brenner for assistance and the referee for his comments. G. M. is supported by an EMBO Fellowship.

Received April 1; accepted May 12, 1975.

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letters to nature

Possible models for some transient X-ray sources

A TRANSIENT X-ray source flared up in Centaurus during December 1974 and was observed by the satellite Ariel V in the energy interval 3–30 keV (refs 1 and 2). Within a few days the source increased in intensity to a peak of $0.02 \text{ photons cm}^{-2} \text{ s}^{-1} \text{ keV}^{-1}$. After reaching a maximum, the intensity decayed with an e -folding time of about 7 d. About one month after the first detection the intensity had fallen by a factor $\gtrsim 5$. No further observations have yet been reported.

The significance of this source is that it exhibits a definite periodicity, P , of $6.75 \pm 0.03 \text{ min}$. This may shed some light on the nature of the object and (possibly) of other transient sources as well. We explore here two alternative models for this phenomenon and suggest in both cases that the source may flare again in the near future; although there is no clear evidence for repetitive outbursts, repetitive behaviour cannot be ruled out (it is, indeed, suspicious that several transient X-ray sources have been discovered in Centaurus³).

In the first model we ascribe the periodicity, P , of 6.75 min to the intrinsic rotation of a compact star such as a white dwarf or a neutron star (no periodicity is expected for a rotating black hole, which is axisymmetric). If the compact star (an X-ray pulsar) moves in an eccentric orbit about a more massive, slightly evolved star, and accretes mass from the stellar wind emanating from that star, the accretion rate is time dependent. The X-ray luminosity can then exhibit large (periodic) changes, provided that it remains far below the Eddington limit.

We assume that the primary (mass, M ; radius, R) loses mass in the form of a steady wind with a velocity, w , of the order of the escape velocity, v_{esc} :

$$w = (2GM/R)^{1/2} = 6.2 \times 10^7 (M/10 M_{\odot})^{1/2} (R/10 R_{\odot})^{-1/2} \text{ cm s}^{-1} \quad (1)$$

The luminosity, L , resulting from accretion on to a compact star of mass M_x and radius R_x is given by

$$L = (GM_x/R_x) \dot{M}_x \quad (2)$$

where $\dot{M}_x = \lambda \pi R_A^2 w \rho$ is the accretion rate, R_A is the accretion radius given by $R_A = GM_x/w^2$, ρ is the stellar-wind mass density at the compact-star orbit, and λ is a constant of the order of unity (for sub-Eddington accretion). We neglect the orbital velocity of the compact object in comparison with the wind speed, which is appropriate whenever $w \simeq v_{\text{esc}}$. As the wind density falls with the square of the distance from the primary, we can relate the rate of accretion to the rate of loss of mass, $\dot{M}$, of the main star, and obtain

$$L = (GM_x/R_x)(\lambda/4)\dot{M}(R_A/r)^2 \quad (3)$$

As the observed ratio of luminosities is $\gtrsim 5$, the ratio between perihelion and aphelion distances must satisfy

$$(L_{\text{max}}/L_{\text{min}}) = (r_{\text{max}}/r_{\text{min}})^2 = [(1+\epsilon)/(1-\epsilon)]^2 \geq 5 \quad (4)$$

The eccentricity, ϵ , should, therefore, be ≥ 0.4 . Such a value is quite reasonable: the eccentricity induced in a close binary

system by a supernova explosion which leaves a neutron star can easily exceed 0.4 (ref. 4). Moreover, the binary pulsar PSR1913+16 has an eccentricity of 0.615 (ref. 5).

The orbital period P , must certainly be longer than the observed lifetime, τ , of the source $\approx 1 \text{ month}$. Using Kepler's third law we, therefore, require

$$a = \left[\frac{G(M+M_x)}{4\pi^2} \right]^{1/3} P^{2/3} > 6 \times 10^{12} \left(\frac{M}{10 M_{\odot}} \right)^{1/3} \left(1 + \frac{M_x}{M} \right)^{1/3} \left(\frac{\tau}{1 \text{ month}} \right)^{2/3} \quad (5)$$

where a is the orbital semimajor axis.

In order that the luminosity remain below the Eddington limit along the entire orbit, the rate of loss of mass, $\dot{M}$ must satisfy

$$\dot{M} < 3 \times 10^{-5} \lambda^{-1} \left(\frac{r_{\text{min}}}{R} \right)^2 \left(\frac{M}{10 M_{\odot}} \right)^2 \left(\frac{M_x}{M_{\odot}} \right)^{-2} \left(\frac{R_x}{10 \text{ km}} \right) M_{\odot} \text{ yr}^{-1} \quad (6)$$

Low energy absorption below 4 keV indicates that the X rays traverse a column density of roughly $6 \times 10^{22} \text{ atoms cm}^{-2}$. If this absorption occurs in the wind itself, we have

$$\frac{\rho}{m_H} r_{\text{min}} = \frac{\dot{M} r_{\text{min}}}{4\pi R^2 w m_H} \left(\frac{R}{r_{\text{min}}} \right)^2 \simeq 6 \times 10^{22} \text{ cm}^{-2} \quad (7a)$$

which, together with equation (6) implies

$$r_{\text{min}} > 2.5 \times 10^{10} \lambda \left(\frac{M}{10 M_{\odot}} \right)^{-3/2} \left(\frac{R}{10 R_{\odot}} \right)^{3/2} \left(\frac{M_x}{M_{\odot}} \right)^2 \left(\frac{R_x}{10 \text{ km}} \right)^{-1} \text{ cm} \quad (7b)$$

Finally, the observed e -folding time for decay of the luminosity implies that $r_{\text{min}}/v_{\text{min}} \sim 7 \text{ d}$, where the orbital velocity at perihelion is given by $v_{\text{min}}^2 = G(M+M_x)(1+\epsilon)/r_{\text{min}}$. Therefore, as $0.4 < \epsilon < 1$,

$$r_{\text{min}} \simeq 9 \times 10^{12} \left(\frac{M}{10 M_{\odot}} \right)^{1/3} \left(1 + \frac{M_x}{M} \right)^{1/3} \text{ cm} \quad (8)$$

All of these restrictions on the model parameters are mutually consistent. It seems possible, therefore, that the model applies to the transient source observed by Ariel V, and perhaps to similar sources as well. It is, unfortunately, impossible—with the data available at present—to make a definite prediction for the date when the source should 'reoccur'. Further monitoring of the same region, however, should confirm this model or make it more and more implausible. The present error in the determination of P ($\pm 0.03 \text{ min}$) precludes the measurement of the orbital Doppler shift if $v_{\text{min}} < 10^3 \text{ km s}^{-1}$. Higher accuracy would specify further the orbital parameters. The symmetry in the luminosity behaviour before and after the peak will be distorted by different amounts of absorption. Though it cannot be said whether the compact source is a white dwarf or a neutron

star, the latter would be more plausible in view of the required eccentricity and the emitted, hard, X-ray spectrum. (On the other hand the very slow rotation is more typical of a white dwarf.)

A second possible model attributes the 6-min periodicity to the orbital period of a binary system containing a compact object. In this case, equation (5) implies

$$a = 2 \times 10^{10} \left(\frac{M}{10M_{\odot}} \right)^{1/3} \left(1 + \frac{M_x}{M} \right)^{1/3} \text{ cm} \quad (9)$$

The orbit will decay because of the emission of gravitational radiation in a time scale, τ , given by

$$\begin{aligned} \tau &= \frac{P}{\dot{P}} = \frac{10(2\pi)^{8/3} c^5}{192} \cdot \frac{(1 + M_x/M)}{M^{2/3} M_x P^{8/3}} \\ &= 1.7 \times 10^4 \left(\frac{M_x}{M_{\odot}} \right)^{-1} \left(\frac{M}{10M_{\odot}} \right)^{-2/3} \left(1 + \frac{M_x}{M_{\odot}} \right)^{1/3} \text{ yr} \end{aligned} \quad (10)$$

where we assume a circular orbit (see ref. 6).

The compact object undergoing accretion can be any kind of collapsed object in this case. The primary must be small ($R < a$) and undergo irregular mass loss. The X-ray emission would then exhibit sporadic behaviour corresponding to the erratic occurrence of loss of mass. The accretion rate could approach the Eddington critical value in this situation and no restriction need be placed on the rate of loss of mass from the primary during an outburst.

Irregular loss of mass amounting to $M \sim 10^{-7} M_{\odot} \text{ yr}^{-1}$ is observed from some small primary stars in compact binary systems, which show occasional eruptive behaviour at optical wavelengths. It is easy to see that this could produce a strong X-ray source with our orbital parameters. If, indeed, transient X-ray sources are identified with known types of eruptive binaries, typical time scales for reoccurrence would be of the order of months, but no definite periodicities should be expected. One may, however, expect changes in the orbital period because of gravitational radiation and mass loss itself⁷.

F. P. acknowledges the hospitality of the Center for Radiophysics and Space Research, Department of Astronomy, Cornell University, where this work was completed. The work was supported by NATO and the National Science Foundation.

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direct connection between the arrival directions and the source directions at those energies. Above a few times 10^{17} eV, however, protons coming from galactic sources should be increasingly anisotropic¹. Comparison of the calculated and observed distributions²⁻⁴ shows that if the primaries are protons then they should be predominantly extragalactic between a few times 10^{17} and 10^{19} eV (ref. 5). More recent data in this energy range^{6,7} do not change this conclusion. At 10^{19} eV the Larmor radius of a proton in the Galaxy is about 3 kpc, much larger than the scale height of the magnetic field in most conventional models^{1,8} and an isotropic distribution above that energy would almost certainly imply a predominantly extragalactic origin if the primaries of the extensive air showers detected are mainly protons. What evidence there is about the mass composition is suggestive of at least some of the very energetic primaries being protons^{9,10}.

The arrival directions of the 87 largest air showers ($E > 1.5 \times 10^{19}$ eV) available in 1974 were analysed by Linsley and Watson¹¹ and the distribution found to be isotropic. Very recently, however, the data have been increased by 20 showers from the Yakutsk array with estimated energy $\geq 10^{19}$ eV (ref. 12). These seem to show some clustering in right ascension. In a joint paper of the Haverah Park, Volcano Ranch and Yakutsk groups¹³ these 20 showers and 12 showers with $10^{19} < E < 1.5 \times 10^{19}$ eV from Haverah Park have been added to the previous set, bringing the total world statistics to 119, and it is claimed that there is *prima facie* evidence for a huge anisotropy. The anisotropy has been tentatively interpreted in terms of an extragalactic origin. In a subsequent paper by Hillas and Ouldrige¹⁴, however, it is claimed that the "observed anisotropies" give strong support for a galactic origin of cosmic rays.

We wish to sound a note of caution on two grounds: on the weakness of the evidence for the anisotropy; and on the ambiguities of the interpretation even if the 'observed' anisotropies reflect a genuine effect.

In a recent survey¹⁵ a detailed account of the statistical aspects of the anisotropy proposed by Krasilnikov *et al.* has been given. Without going into the details, we reiterate here some of the main points. The impressive significance levels published by Krasilnikov *et al.* represent to some extent *a posteriori* statistics both from the point of view of choosing the method of harmonic analysis in right ascension (instead of a χ^2 analysis, analysis of high maxima in individual bins or checking whether there is some intensity increase in directions suggested by model calculations) and because there was some measure of selection of those declination intervals where either the first or the second harmonic has a fairly high significance. Kiraly and White have reanalysed the data by the three methods mentioned above and have found no strong indication for anisotropy (the significance levels were some tens of per cent instead of the order of 1% as shown by Krasilnikov *et al.*). The validity of the harmonic method rests on the predominance of low order spherical harmonics in the angular distribution, and this is not borne out even *a posteriori* by the fairly sharp apparent intensity peaks.

As far as the suggestions of Hillas and Ouldrige are concerned, we agree that a galactic origin in the whole energy range would have some attractive features. But the directions of the three intensity maxima show no obvious association with either the plane or centre of the Galaxy. It is true that one of the three peaks is in the region of zero galactic longitude with latitudes in the range 0 to -90° and this peak, as proposed by Hillas and Ouldrige, may be caused by a flux coming from the direction of the galactic centre and bent by a hypothetical large scale magnetic field. But there are two objections: the field required is oppositely directed to that observed locally; and, when energies are assigned to the showers, it is found that those of highest energy in this peak seem to cluster away from the galactic centre rather than in its vicinity.

Even if the large scale distribution of the arrival directions shows no significant features, some point sources might still emerge at the highest energies, giving rise to angular correlation

Origin of ultra high energy cosmic rays

THE origin and propagation of cosmic rays have been intriguing physicists ever since their discovery in 1912. The main obstacle to identifying the sources of charged particles is the galactic magnetic field. Since the Larmor radii for protons in a typical interstellar field of 3 μ gauss are below 30 pc (the typical scale for field irregularities) for $E < 10^{17}$ eV, there should be no

between the showers and the source candidates. We have carried out a comprehensive search for such features. Although no strongly significant correlations have been found, there is some statistical indication for an enhanced intensity around the directions of QSOs, extragalactic supernova explosions and galactic supernova remnants. Confronting physical plausibility with the statistical evidence, however, statistical fluctuations seem to be the most likely explanation, just as in the case of the large scale anisotropies proposed by Krasilnikov *et al.*

So the recent indications for large and small scale anisotropies in the directional distribution of extremely high energy particles are not significant enough to be considered as a strong argument in the controversy about galactic versus extragalactic origin. We hope, however, that these indications will give further impetus to the enhancement of both the quality and quantity of the experimental results, which have already shown much improvement in the past few years.

The authors are grateful to the Science Research Council for its support and to Dr A. A. Watson for helpful elucidations.

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Transverse fault systems in fold belts and oceanic fracture zones

THE fracture zones of modern oceans seem to be the favoured channelways for the upwelling of mineralised hydrothermal fluids¹⁻³. It is proposed that deeply penetrating, transverse fault zones in the Caledonian and other fold belts were derived from such oceanic fracture zones, and continued to act as channelways for the uprising of magma and mineralised thermal waters throughout the evolution of the oceanic trough, during orogenesis and even into post-collision (post-orogenic) times. The collated evidence suggests that such fault systems are widespread in fold belts of various ages.

Oceanic fracture zones (including transform faults) in oceanic troughs lie parallel to the direction of rift opening and to the direction of subsequent widening of rifts. These fracture zones provide fundamental constraints on plate motions and are subparallel and directly related to pre-existing lines of weakness in the fragmented continental blocks. It follows that in all continental blocks alongside modern oceans with regular fracture zones of this type (and this seems to be the great majority) there are systems of subparallel lines of weakness.

It also follows that the tensional stresses which lead to rifting and to the separation of parts of these continental blocks must operate parallel to these lines of weakness. This is directly borne out, for example, by the relationship of transverse fracture zones to the Red Sea Rift¹ which is regarded as a type example of incipient continental rifting by seafloor spreading. Here, there is a close spatial relationship between the distribution of transform faults and metallisation¹ and Bignell¹ has suggested that the faults permitted metal-scavenging brines to pass through Miocene evaporites and to discharge into brine pools along the median ridge. Similar abnormal metal concentrations, overlying the median ridge or fracture zones in the axial areas of the Pacific² and Atlantic³ oceans have, however, been related to volcanic, hydrothermal (hot spring) activity.

Horne^{4,5} has proposed that the distribution of metallisation in the Caledonian Fold Belt of Ireland and Britain is controlled by a system of transverse fault zones which are quite regularly spaced, subparallel and consistently normal to or at a very high angle to the regional structural strike. These transverse fault zones were also channelways for uprising of magma during the Devonian and Lower Carboniferous and provided pathways for Tertiary feeder dykes; they were, and still are, channelways for rising thermal waters. They therefore seem to be features of great vertical and lateral penetration and temporal persistence.

A number of similar transverse fault systems have been recognised in other fold belts. From studies of ERTS photography Lathram *et al.*⁶ recognised two intersecting sets of regional lineaments in Alaska, trending nearly north-east (transverse) and north-west (strike). The north-easterly trending cross-faults are directly comparable with the transverse fault system in the Caledonides in regard to their scale, their linearity or gentle sinuosity and their transverse relationship to the fold belt.

A similar series of transverse faults has been recognised⁷ in western Canada. The most interesting and well documented of this set is the major ENE-striking McDonald-East Arm Fault. Lead-zinc metallisation, including the massive Pine Point deposit, extends for at least 55 km along the strike of this transverse zone. The cross-strike reef barrier of the Presquile Formation, which is the dolomitised host rock, formed as a narrow reef development along the fault scarp^{7,8}.

In the western US-Canadian border area east-west patterns occur in the distributions of mineral deposits and Mesozoic to Cainozoic igneous rocks⁹. Hodgson⁹ inferred the existence in that region of a series of transverse faults normal to the local north-south trend of the Western Cordillera which influenced facies patterns in sedimentary rocks dating from the late Precambrian to the mid Palaeozoic. Hodgson has suggested a relationship between these transverse faults and continentward extensions of transform faults formed in a late Precambrian, proto-Pacific ocean.

Hoppin¹⁰ has described a set of at least five lineaments from the Bighorn Mountains of Wyoming, which are transverse to the regional structural trend, are either straight or gently curved in plan, have regular spacing, show little evidence of strike-slip movement and have a close spatial association with thermal springs and metallisation.

The Czechoslovakian epigenetic ore deposits, notably the major polymetallic sulphide deposits at Příbram, Jihlava, Jáchymov, Kutná Hora and Jeseník are aligned along a series of regularly spaced transverse fault zones striking NNE in the Czechoslovakian part of the Bohemian Massif¹¹. Thermal and mineral springs, including those at Karlovy Vary (Carlsbad) and Jáchymov, also coincide with the traces of these transverse fault zones.

Horne^{4,5} has proposed that the transverse fault system of the Caledonian Fold Belt of Ireland and Britain is directly related to a system of oceanic fracture zones generated in the spreading phase of the Caledonian Ocean, and to their

associated continental fracture lines. This implies that during closure of the Caledonian Ocean by crustal subduction the fracture zones continued to constrain relative movements between plates. The zones then continued to act as fundamental lines of weakness and differential crustal adjustment during continental convergence and compression of the ocean fill.

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Medical radionuclides in marine environment

RADIOACTIVE discharges resulting from the practice of nuclear medicine are controlled by the Department of the Environment, who grant licences to individual hospitals for the discharge of liquid wastes under the Radioactive Substances Act, 1960. A few years ago most workers in nuclear medicine would have thought that the large degree of dilution occurring when hospital effluent enters a public sewage system would remove any possibility of it having any environmental effect. Because of the recent rapid increase in the medical use of radionuclides, however, there is now some concern regarding environmental contamination^{1,2}.

It seems that the radionuclides of greatest importance in this regard in the Swansea area are iodine-131 and technetium-99m. ¹³¹I is regularly used for the treatment of thyrotoxicosis in activities of several millicuries per patient and is also occasionally used in quantities of up to 200 mCi for the treatment of a patient suffering from thyroid cancer. The short lived ^{99m}Tc (half life 6.0 h) is chiefly used as a body organ imaging agent in activities of around 10 mCi per investigation, and is the radionuclide which has shown the greatest increase in usage during the past few years.

After entering the public sewage system, radioactive wastes from Swansea hospitals pass through a mechanical screening

and maceration process and are then discharged into the sea. The degree of dilution is therefore considerable, but on the other hand the published concentration factor^{3,4}, for various radionuclides in seaweeds are also very large. Data supplied by D. F. Jeffries of the Fisheries Radiobiological Laboratory suggest concentration factors of up to 10⁴ for ¹³¹I in marine algae and much greater concentration factors for ^{99m}Tc. These concentration factors result from experiments in which the long term steady-state equilibrium has been reached. Because of the importance of short term effects resulting from nuclear medicine practices, experiments were carried out on the dynamic uptake of ¹³¹I and ^{99m}Tc by the common seaweeds *Fucus serratus*, *Laminaria digitata*, *Ulva lactuca* and *Porphyra umbilicalis*. Specimens of seaweed were collected and placed in polythene containers holding seawater to which a few microcuries of the radionuclide under test had been added. The water temperature was held

Table 1 Radioiodine content of some common seaweeds in Bracelet Bay, June–October 1974

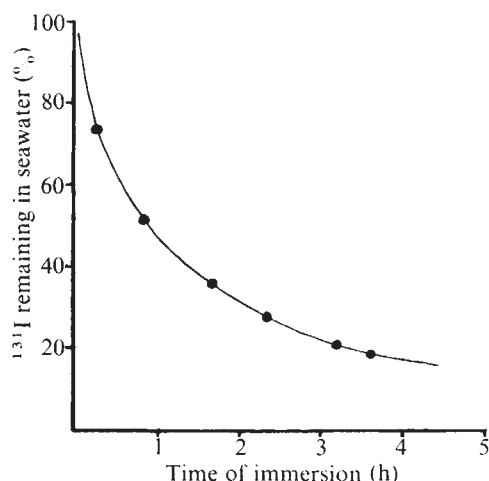
Date	Species	Estimated ¹³¹ I content, pCi kg ⁻¹
June 21	<i>Fucus serratus</i>	123
June 21	<i>Ulva lactuca</i>	78
July 19	<i>Fucus serratus</i>	208
October 24	<i>Fucus serratus</i> (3 samples)	37 41 68
October 25	<i>Fucus serratus</i> (3 samples)	500 540 697
October 25	<i>Porphyra umbilicalis</i>	446
October 27	<i>Fucus serratus</i>	482
October 27	<i>Porphyra umbilicalis</i>	231

at 10 °C to simulate sea conditions. Measurements of radioactivity were made in a large 'bin counter', which consists of a ring of eight Geiger tubes (type G26PB) arranged around a cylindrical sample volume. Lead shielding (8 cm thick) surrounds the apparatus to reduce background counts. The radioactivity in the seaweed was determined by first placing the whole container in the counter, then removing the seaweed sample and taking the difference in counts registered on a scaler in 100 s.

The avidity of all these seaweeds for the two radionuclides was remarkable and is illustrated for *Fucus serratus* in Figs 1 and 2, which have been corrected for radioactive decay. It is apparent that these radionuclides will not remain free in seawater for any great length of time, but will rapidly be taken up by any seaweeds which are present.

Because of these results I decided to measure the ¹³¹I content of seaweed specimens taken from beaches near the sewage outfall, with samples of the same species taken from remote beaches as background comparisons. For these measurements a NaI (Tl) crystal detector 12.5 cm diameter × 7.5 cm thick was used, surrounded by a minimum of 5 cm of lead shielding. A sample volume of 0.5 l was available into which a polythene container holding approximately 250 g of seaweed could be placed. Seaweed samples were weighed, cut into small pieces and packed into a volume of 450 ml in the container. Water was then added to fill any spaces, thus ensuring reproducible geometry and self-absorption in the specimen. A single channel pulse-height analyser was used with a window of 306–404 keV and the output pulses were counted on an autoscaler. Counting times were usually 10⁴ s and in this time background counts of about 17,500 would be obtained with no sample present. The minimum detectable concentration of ¹³¹I in water (which gives an increase of three times the standard deviation of the

Fig. 1 Extraction of ¹³¹I (sodium iodide) from 900 ml seawater by 31.7 g of *Fucus serratus*.



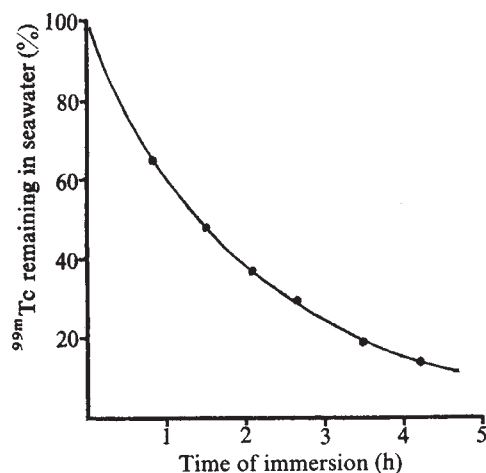


Fig. 2 Extraction of ^{99m}Tc (pertechnetate) from 900 ml seawater by 25 g of *Fucus serratus*.

background⁵, in 10^4 s) is 16.5 pCi in a volume of 450 ml, that is 36.7 pCi l⁻¹.

With 250 g seaweed samples from remote beaches in the detector around 18,500 counts were recorded in the same counting time. The increase must be attributed to the presence in the sample of naturally occurring potassium-40 and of other radionuclides resulting from fallout or nuclear power generation. Because the level of this background activity was somewhat variable from sample to sample I decided to take the highest value found in several samples from remote beaches as the "natural background" for any species. Only when samples taken from near the sewage outfall showed activities which were significantly higher than this could any contribution from the presence of medical radionuclides be assumed.

Table 1 shows some higher than usual results obtained during the period June to October 1974, when occasional monitoring was carried out. Radioactive decay studies carried out on the samples collected in June and July showed a decay rate (above the relatively constant natural background level) which was compatible with the 8 d half life of ^{131}I . The two samples of *Fucus serratus* collected on October 25 which contained the highest amounts of activity were sent to H. C. Biggin of Sheffield Polytechnic, who carried out high resolution gamma ray spectrometry using a lithium-drifted germanium semiconductor detector. This showed conclusively that ^{131}I was present in these samples.

The most active samples were obtained at low tide from Bracelet Bay, which is approximately 600 m west of the outfall. Samples taken from other nearby sites often showed no significant activity above the natural background, even though some were taken from places nearer the outfall. The distribution of radioactivity along the shore is variable, as may be expected since the effluent from the outfall is subject to the varying effects of wind and tidal conditions. It is therefore not certain that the site of maximum radioiodine content has ever been located, because of the limited number of samples it has been possible to obtain. The Oceanography Department of the University College of Swansea, is now studying the transport of effluent from the outfall under the influence of near-shore currents.

The greatest concentration of radioiodine found in *Porphyra*, which is collected on these beaches and eaten locally as 'laverbread', was 446 pCi kg⁻¹. A market survey by Preston and Jeffries⁶ showed that the heaviest consumption of laverbread by a member of the public was 388 g d⁻¹. Assuming that this quantity was eaten by an individual who

gathered *Porphyra* in Bracelet Bay and that no radioiodine washes out during cooking, his maximum daily intake of ^{131}I would be 173 pCi. The maximum permissible intake for adult members of the public⁷ is 1.6 $\mu\text{Ci yr}^{-1}$, equivalent to 4,384 pCi d⁻¹. Thus the levels of radioiodine found in measurements so far represent at most only 4% of the recommended limit.

It is reassuring to have found such a large safety margin, but it seems necessary to continue these measurements, particularly at periods when large activities of radioiodine are administered to patients. Also, because of the rapidly increasing medical use of ^{99m}Tc it seems of interest to determine if measurable levels of this radionuclide occur in seaweed. It is not likely, however, that any health hazard will result from use of this material, since it has a short half life and low radio-toxicity.

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Received April 9; accepted May 6, 1975.

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Plastic films on the bottom of the Skagerack

FISHERMEN from Smögen, Sweden have remarked that when they trawl along the bottom of the Skagerack they almost invariably collect sheets of plastic in their nets.

Some of these films (Fig. 1) have been analysed, using infrared spectroscopy (IR) and gel permeation chromatography (GPC), (see ref. 1). The films were found at depths varying between 180 and 400 m.

The analyses show that the films consist of low density polyethylene (LDPE) which shows evidence of the pronounced degradation typical in LDPE exposed to sunlight in the presence of air and water. The films therefore become rather brittle. They are also partly covered with biological material and in some cases they show what can be described as 'eating traces' (Fig. 1).

The biological material is apparently a kind of calcareous bryozoan, and is present in amounts which correspond to a growth time of 3-4 months. This group of animals commonly occurs at depths of up to 15 m. Furthermore, there are also traces of the brown alga *Lithoderma*, in amounts which also correspond to 3-4 months of growth. *Lithoderma* grows at depths of 15-25 m.

The fact that films of LDPE have been discovered at great depths, although they originally had a density of 0.92 g cm⁻³ and, consequently, should have floated on water, is both surprising and serious. It has been presumed previously that this type of material remains afloat and probably finally reaches land.

I can, however, suggest a possible hypothesis to explain these findings. First, the material floats for a while on the surface where it is exposed to sunlight so that it becomes degraded. At the same time, the bryozoa begin to grow on the material, weighing it down so that it slowly sinks towards the pycnocline. The material stops at this level and *Lithoderma* starts to grow on it. Eventually the film will weigh enough to sink to the very bottom.

The 'eating traces' are probably caused by plant-eating molluscs which consume *Lithoderma* and which ingest the underlying film at the same time.

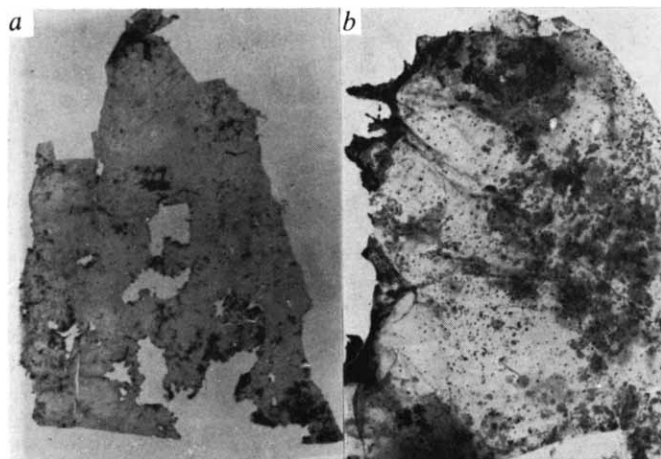


Fig. 1 *a*, Severely degraded thin film showing 'eating traces' and growing material; *b*, less degraded, thick film with a large amount of growing material. The black dots and threads are Lithoderma; the grey areas are bryozoans. Area shown: 25×40 cm in both cases.

The investigation presented here stresses a problem which arises when plastics are thrown into the sea from ships. Plastics are used on ships as cargo wrappings and as ordinary plastic bags. Large sheets, thrown overboard, are not only a potential hazard to the propellers of smaller ships, but also cause trouble to fishermen who get them into their trawls. Furthermore, the films must also be considered as a source of pollution.

Our samples were provided by the crews of the LL 910 Hållö and LL 321 Sandvik, both from Smögen.

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Received April 11; accepted May 5, 1975.

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Lattice absorption in small particles

KREIBIG and Fragstein¹ carried out experiments on small silver particles which showed a strong dependence of some optical properties on the particle radius. They were able to explain their results by assuming that electrons made collisions with the walls of the particle, the mean free path being equal to the particle radius.

Here I suggest that a similar effect may occur with the behaviour of phonons. With phonons in small particles of radius R we may associate an additional collision time of order, in this case, $\tau \sim R/v$, where v is the velocity of sound in the bulk material. If such an effect occurs an obvious means of detecting it would be in the lattice absorption of ionic solids. Ipatova, Maradudin and Wallis² have given a theoretical treatment of lattice absorption in NaCl and LiF in which they show the finite width of the absorption peak to be the result of anharmonic contributions to the potential. For NaCl they find that the damping constant γ and angular frequency of the peak ω_0 are related to the temperature and characteristic temperature by

$$\gamma/\omega_0 \sim 0.041/\Theta + 0.05(I/\Theta)$$

where $\Theta \sim 370$ K, $\omega_0 \sim 3 \times 10^{15}$ s⁻¹ for NaCl. For particles of radius $< 10^{-6}$ cm and $T < 10$ K, taking $v \sim 5 \times 10^5$ cm s⁻¹, the

effect I suggest should be easily detectable—that is, the width of the lattice absorption peak will be dominated by the collisions of phonons with the walls of the particle.

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Received April 24; accepted May 6, 1975.

¹ Kreibig, U., and Fragstein, C. V., *Z. Phys.*, **224**, 307 (1969).

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Lone electron pairs and stereochemistry

THE two basic assumptions of the Nyholm-Gillespie Theory^{1,2} are, first, that valence shell electron pairs repel one another, the system adopting a minimum repulsion energy configuration (condition (i)), and second, that lone-pair-lone-pair (LL) repulsions are greater than lone-pair-bonded-pair (LB) repulsions which are, in turn, greater than bonded-pair-bonded-pair (BB) repulsions (condition (ii)). To offer a full explanation of the gross stereochemistry of all octahedral and pyramidal complexes two additional axioms are necessary: first, that lone-pair-bonded pair repulsions are less than the arithmetic mean of the lone-pair-lone-pair and the bonded-pair-bonded-pair repulsions (condition (iii)) and second, that electron pair repulsions at 120° to each other are less than half the corresponding repulsions at 90° to each other (condition (iv)). The four rules together enable a rationalisation of the stereochemistry of all known octahedral and bipyramidal complexes.

The rationale behind these assumptions can be illustrated by considering four important cases:

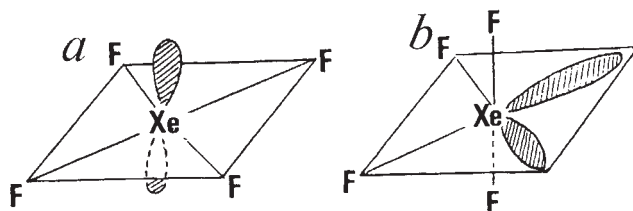
The case of octahedral coordination, with two lone pairs as exemplified by XeF₄, ICl₄⁻, and so on. Experimentally, the *trans*-arrangement of lone pairs is more stable than the *cis*-arrangement (Fig. 1*a* and *b*), and the systems are square planar. In considering valence shell repulsions electron pair interactions at 180° to each other can be ignored, since they can exert no component of force perpendicular to the bond axis.

In the *trans*-arrangement (Fig. 1*a*) there are four bonded-pair-bonded-pair interactions and eight lone-pair-bonded-pair interactions, all at 90° (4BB+8LB) whereas in the *cis*-arrangement (Fig. 1*b*) there are five bonded-pair-bonded-pair interactions, six lone-pair-bonded-pair interactions and one lone-pair-lone-pair interaction, all at 90° (5BB+8LB+LL). In order that the *trans*-arrangement should be more stable than the *cis*-arrangement it is necessary that condition (iii) is fulfilled:

$$LB < (BB + LL)/2 \quad (1)$$

The case of trigonal bipyramidal coordination, with one lone-pair as exemplified by SF₄, SeCl₄, and so on. Two possible arrangements arise in this case, the first has C_{2v} symmetry,

Fig. 1 Octahedral coordination with two lone pairs, exemplified by XeF₄: *a*, *trans*-arrangement; *b*, *cis*-arrangement.



the second C_{2v} symmetry (Fig. 2a and b). All known examples of this coordination possess the C_{2v} structure (Fig. 2a).

For the undistorted bipyramid there are three relevant angles: 90° , 120° and 180° . As in the case already considered the interactions at 180° can be ignored; however, interactions at 120° can be either neglected completely at a fairly low level of sophistication or can be assumed to be a fraction β , which is itself less than unity, of the corresponding interactions at 90° . In the first case C_{2v} - SF_4 has four bonded-pair-bonded-pair interactions and two lone-pair-bonded-pair interactions ($4BB+2LB$) and C_{3v} - SF_4 has three of the former interactions and three of the latter ($3BB+3LB$). If the C_{2v} structure is more stable than C_{3v} , the inequality, $BB < LB$ must hold and is true because of Nyholm and Gillespie's² second condition. In the more sophisticated consideration, which includes interactions not only at 90° but also at 120° , for C_{2v} - SF_4 the total interactions are $[4BB+2LB+\beta(BB+2LB)]$ and for C_{3v} - SF_4 they are $[3BB+3LB+3\beta BB]$. The condition that C_{2v} is more stable than C_{3v} leads to the requirement that:

$$[(1-2\beta)BB] > [(1-2\beta)LB] \quad (2)$$

which is true provided that $\beta < 0.5$; otherwise $BB > LB$ which violates the second basic assumption of the Nyholm-Gillespie² theory. In consequence of this inequality it is necessary to postulate electron pair interactions at 120° to each other are less than half the corresponding interactions at 90° to each other (condition (iv)).

The case of trigonal bipyramidal coordination with two lone-pairs as exemplified by ClF_3 , BrF_3 , SCl_3^+ and so on. Three possible arrangements arise in this case: D_{3h} , C_{2v} and C_s , according to the point group of the molecule (Fig. 3). For interactions at 90° , the total number of interactions in the three cases are as follows: D_{3h} ($6LB$), C_{2v} ($2BB+4LB$) and C_s ($2BB+3LB+LL$). From the Nyholm-Gillespie² condition that $LB > BB$ it follows that the C_{2v} arrangement is more stable than the D_{3h} arrangement; furthermore, since $LB < LL$ it follows that the D_{3h} structure is more stable than the C_s structure. This is the situation in reality. For interactions at 120° in addition to those at 90° , they are, for the D_{3h} case $[6LB+3\beta BB]$, for the C_{2v} case $[2BB+4LB+\beta(2LB+LL)]$ and for the C_s case $[2BB+3LB+LL+\beta(BB+2LB)]$. If the C_{2v} structure is more stable than the D_{3h} arrangement then

$$\left[\frac{(2-3\beta)}{2(1-\beta)} \right] BB + \left[\frac{\beta}{2(1-\beta)} \right] LL < LB \quad (3)$$

And if the C_{2v} arrangement is more stable than C_s then

$$LB < [\beta BB + (1-\beta)LL] \quad (4)$$

Substituting our condition that $LB < [BB+LL]$ into equations (3) and (4) reduces both equations to the inequality:

$$(0.5-\beta)BB < (0.5-\beta)LL \quad (5)$$

which, given the second Nyholm-Gillespie condition (ii) that $BB < LL$, will be true so long as $\beta < 0.5$ (condition (iv)).

Fig. 2 Trigonal bipyramidal coordination, with one lone-pair, exemplified by SF_4 : a, C_{2v} arrangement; b, C_{3v} arrangement.

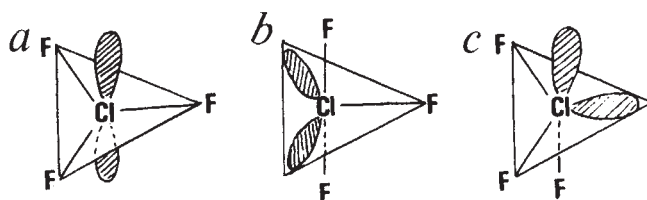
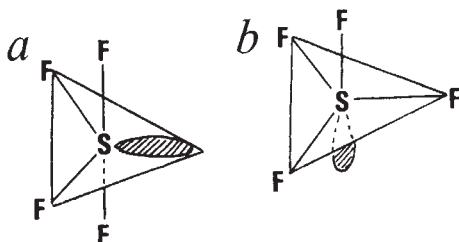


Fig. 3 Trigonal bipyramidal coordination, with two lone-pairs, exemplified by Cl_3F : a, D_{3h} arrangement; b, C_{2v} arrangement; c, C_s arrangement.

The case of trigonal bipyramidal coordination with three lone-pairs as exemplified by XeF_2 , ClF_2^- , and so on. Three possible structures arise, two having C_{2v} symmetry, the third having D_{3h} symmetry, corresponding respectively to an undistorted XeF_2 structure having $FXeF$ angles of 90° , 120° and 180° (Fig. 4 a, b, c, respectively).

Consider interactions at 90° . For structure a, and C_{2v} symmetry, the interactions are ($BB+3LB+2LL$); for structure b, with D_{3h} symmetry, we have ($6LB$); and for the structure c possessing C_{2v} symmetry, ($4LB+2LL$).

If structure b is more stable than structure c the inequality:

$$6LB < [4LB+2LL] \quad (6)$$

must hold. This is true as a result of condition (ii). Structure b will, however be more stable than structure a only if

$$LB < [BB]/3 + 2[LL]/3 \quad (7)$$

and this inequality, being less restrictive than condition (iii), deduced from the octahedral case, holds and predicts that

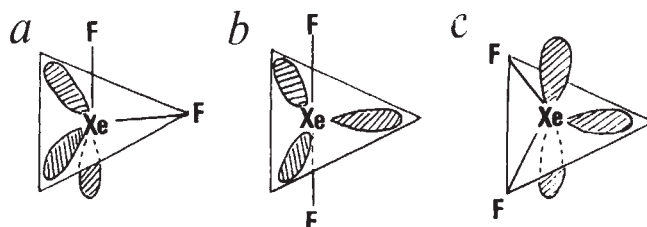


Fig. 4 Trigonal bipyramidal coordination, with three lone-pairs, exemplified by XeF_2 : a, C_{2v} arrangement 90° ; b, D_{3h} arrangement; c, C_{2v} arrangement 180° .

structure b is the most stable arrangement. Both XeF_2 and ClF_2^- are linear molecules.

Consider now the interactions at 120° in addition to those at 90° . Those for structure a are $[BB+3LB+2LL+\beta(2LB+LL)]$; for structure b they are $[6LB+3\beta(LL)]$; and for structure c they are $[4LB+2LL+\beta(BB+2LB)]$.

The requirement that structure b is more stable than structure c is that:

$$LB < \left[\frac{\beta(BB)}{2(1-\beta)} \right] + \left[\frac{(2-3\beta)(LL)}{2(1-\beta)} \right] \quad (8)$$

if we impose an upper limit, corresponding to our condition (iii) that $LB = (LL+BB)/2$, inequality (8) reduces to:

$$[BB(1-2\beta)] < [LL(1-2\beta)] \quad (9)$$

which holds if $BB < LL$ (condition (ii)) and β is less than 0.5 (condition (iv)).

The requirement that structure *b* is more stable than structure *a* is that

$$LB < \left[\frac{BB}{(3-2\beta)} \right] + \left[\frac{2(1+\beta)LL}{(3-2\beta)} \right] \quad (10)$$

which subjected to condition (iii), that $LB = (LL+BB)/2$, leads to:

$$[BB(0.5-\beta)] < [LL(0.5-\beta)] \quad (11)$$

which requires conditions (ii) and (iv) for validation.

We conclude, therefore, that if, in addition to the Nyholm-Gillespie normal conditions we impose additionally two further conditions, we thereby extend the predictive power of the theory of electron-pair repulsions and enable rationalisation of the gross stereochemistry of octahedral and trigonal bipyramidal complexes. It remains to examine these conditions for coordination situations where no known examples exist and indeed to rationalise the requirement that β should be less than 0.5; this will be considered in further studies to be published elsewhere.

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Received March 24; accepted May 5, 1975.

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Mössbauer spectroscopy of Greek 'Etruscan' pottery

WE present here an analysis of the iron compounds in both the core and surface 'gloss' of a sherd of Greek 'Etruscan' black glazed pottery, using both scattering and absorption techniques. The Mössbauer effect¹ is used to measure the magnetic hyperfine splitting, determined by the overall magnetic properties and the isomer shift and quadrupole splitting, which are related to the oxidation state and site symmetry at the Mössbauer site. Mössbauer measurements are particularly effective

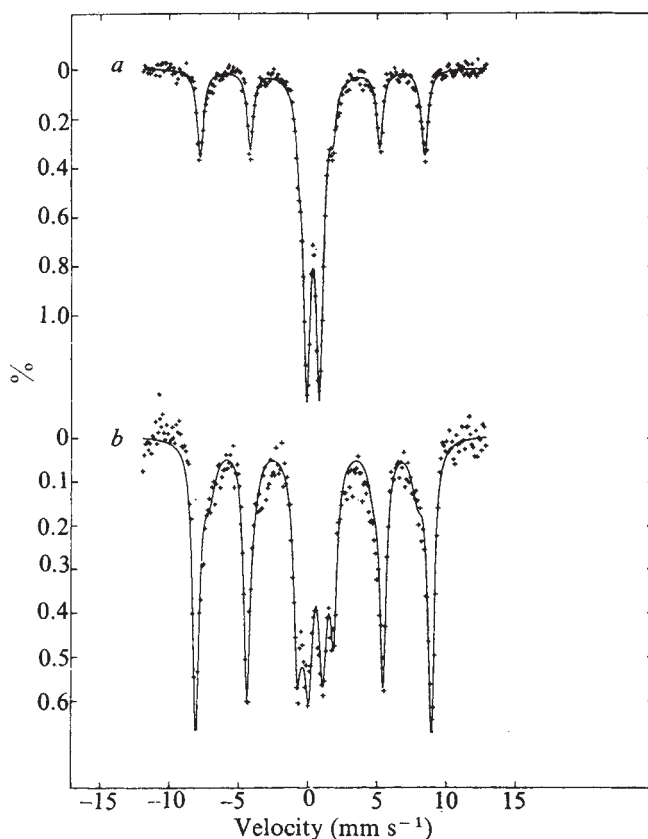


Fig. 1 Mössbauer absorption spectra of the body of a Greek 'Etruscan' potsherd at room temperature (300 K) (a) and at 4.2 K (b). The full lines are least squares fits to the data. In all figures, the observations were obtained using a ⁵⁷Co source in a rhodium matrix.

for structures containing iron, even in finely divided form, and are non destructive.

Early studies of Greek Black have been summarised by Binns and Fraser² who confirmed that control of the firing atmosphere during a single firing could produce a black gloss, attributed to the non-magnetic ferrous oxide (FeO), on a red core of ferric oxide (Fe₂O₃). Bimson³ has outlined a more realistic firing process, and using X-ray diffraction has confirmed the presence of FeO in one sample while no oxides were identified in two other samples. Noble⁴ has suggested that the black colour is caused by 'iron' trapped in a layer of quartz, either as the metal, (Mg,Fe) SiO₃ or as tiny crystals of iron entrapped in quartz. He has also suggested the presence of Fe₃O₄ on the basis of magnetic attraction, whereas Oberlies⁵ has proposed hercynite (FeAl₂O₄) or possibly a member of the series Fe₃O₄–

Table 1 Mössbauer parameters obtained from fitted curves to spectra of (a) the core material (sample 1) and (b) the surface gloss (samples 1 and 2 at 300 K) with the quadrupole splitting (Δ) in mm s⁻¹, the magnetic field (*H*) in koersted, and the widths and shifts in mm s⁻¹. All shifts are quoted with respect to that of α iron

<i>a</i>	Temperature (K)	Magnetically split components (Sextet)					Area	Central doublet		
		Area	<i>H</i>	Shift	Δ	Width		Shift	Δ	Width
Core material	300	36%	502	0.37	0.36	0.58	64%	0.35	0.93	0.65
	4.2	61%	526	0.48	0.23	0.54	22%	0.58	0.99	0.76
		17%	467	0.41	—	1.07				
<i>b</i>	Type of measurement	Magnetically split components (Sextet)				Area	Quadrupole doublets			
		Area	<i>H</i>	Shift	Width		Shift	Δ	Width	
Surface gloss	Transmission	15%	487	0.30	0.60	37%	0.83	1.78	0.68	
	(sample 2)	21%	446	0.68	1.28	27%	0.45	0.53	0.68	
	Transmission	21%	490	0.36	0.65	28%	0.83	1.77	0.70	
	(sample 1)	12%	440	0.67	1.05	39%	0.38	0.63	0.70	
	Scattering (X ray)	22%	499	0.35	0.56	24%	0.80	1.72	0.72	
	(sample 1)	19%	453	0.50	1.41	35%	0.34	0.76	0.60	

FeAl_2O_4 . The structures of the iron compounds responsible for the black colour were not resolved by X-ray diffraction mainly because of the very small particle sizes. This is not normally a serious limitation in Mössbauer spectroscopy, which thus offers a potential solution to the problem.

The sherd examined was a fragment of late sixth century BC Greek 'Etruscan' pottery found at Cerveteri, and was part of an assemblage of fragments signed by Greek artists and potters. It was 0.4 cm thick, both surfaces being black 'glazed' with a typical red core. The iron content of the core and gloss were 5.3% and 6.1% respectively, as determined by neutron activation analysis. The ^{57}Fe Mössbauer absorption spectra of the whole fragment at room temperature and at 4.2 K are shown in Fig. 1. A backscattered Mössbauer spectrum (Fig. 2) of the surface gloss (to a penetration depth of $\approx 12 \mu\text{m}$) was obtained by counting the conversion X rays emitted from the surface layers following Mössbauer absorption of the incident gamma rays. As a comparison, about 10–20 mg of the gloss were removed from this specimen (sample 1) and a similar sherd (sample 2) and the Mössbauer absorption spectra of the latter are shown (Fig. 3) at room temperature, 77 K and 4.2 K. Counting rates in these measurements were about seven times larger than in the scattering measurements while the observed effects were comparable, although only the latter were wholly nondestructive.

The parameters obtained from least squares fits to the spectra are shown in Table 1. The spectra for the core (Fig. 1) consisted of a central quadrupole doublet and an outer sextet resulting from a combined magnetic and quadrupole interaction with the relative area of the doublet decreasing on cooling. This behaviour was caused by the presence of iron as ferric oxide (Fe_2O_3) with an average particle size less than 20 nm, leading to superparamagnetic behaviour⁶. The difference in the quadrupole interaction (Δ) between the doublet and sextet at 300 K,

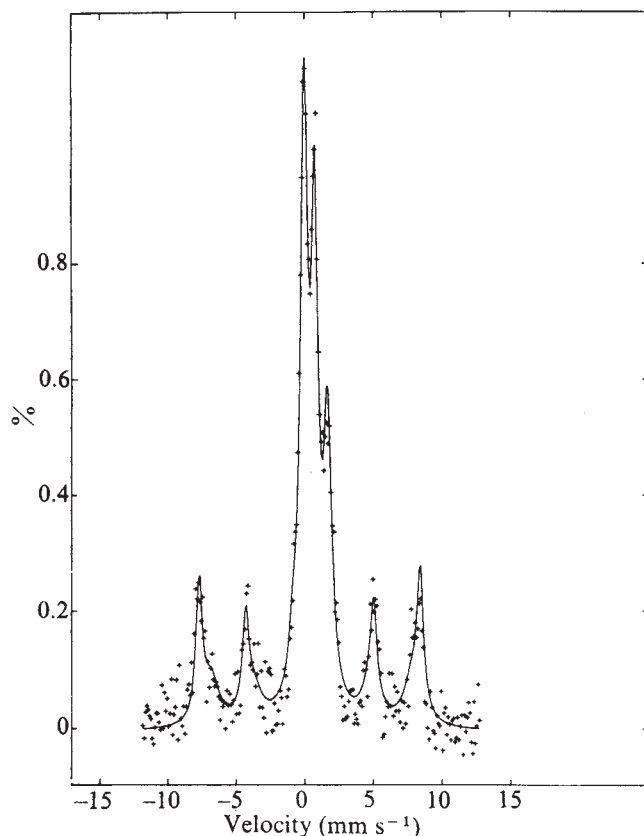


Fig. 2 Mössbauer backscattered (X ray) spectrum of the surface of a Greek 'Etruscan' potsherd at room temperature. The full line is a least squares fit to the data.

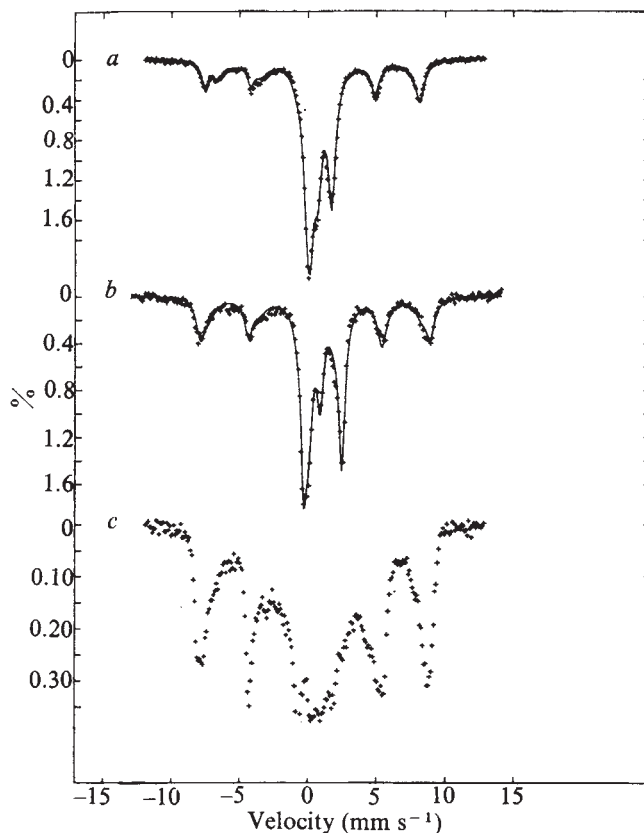


Fig. 3 Mössbauer absorption spectra of material taken from the surface of a Greek 'Etruscan' potsherd (sample 2) at room temperature (a), 77 K (b) and 4.2 K (c). The full lines for the spectra at room temperature and 77 K represent least squares fits.

and the spread in magnetic field (H) at 4.2 K originate from the differences in environment of nuclei situated in the surface and interior of the small particles of ferric oxide⁶.

All spectra for the black gloss at room temperature were analysed in terms of two magnetic sextets and two quadrupole doublets, the results being reasonably consistent. The magnetic constituent seemed to be magnetite (Fe_3O_4) in which the iron atoms occur on two sites. The complex spectrum at 4.2 K was not fitted because of limited statistical accuracy though all the outer magnetic lines were probably caused by ' Fe_3O_4 ', which then has six values of H (six sextets). The values of the relative areas of the inner sextet at 300 K indicate that $\approx 30\%$ of the iron on the octahedral sites has been replaced, for example by aluminium present in the high illite content clay. This would be consistent with a compound at the Fe_3O_4 end of the series Fe_3O_4 – FeAl_2O_4 (ref. 5). The doublet with $\Delta \approx 1.8 \text{ mm s}^{-1}$, is attributed to high spin ferrous ions and the parameters are consistent with those in other silicates⁷ such as the anthophyllite series $(\text{Mg,Fe})_7\text{Si}_8\text{O}_{22}(\text{OH})_2$. The other doublet was attributable to high spin ferric ions, not to FeO . It is significant that the area of each doublet was greatly reduced at 4.2 K because of magnetic splitting, though this is either small or hidden by the outer magnetite components. This suggests that the doublet with $\Delta \approx 0.6 \text{ mm s}^{-1}$ was the result of either finely divided magnetite or possibly ferric ions in a silicate structure as is observed, for example, in the garnet group⁷. Insufficient Mössbauer data are available on the silicates to allow an identification based on the central doublets. This does not invalidate the positive identifications of the iron oxides.

More work is required to see whether these observations are valid for other samples of Greek Black of this period, but the usefulness of the Mössbauer effect as a non-destructive tool for chemical analysis of molecular compositions is clearly

demonstrated. In particular, scattering measurements on surfaces are viable and extend the application of the technique to whole artefacts. The measurements are not limited to those involving iron compounds and we have examined tin compounds in Egyptian and British faience beads and in bronzes.

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Structure of phenolic metabolites of *p,p'*-DDE in rat, wild seal and guillemot

WE recently described the isolation and characterisation of metabolites of polychlorinated biphenyls from field-collected samples, that is faeces from guillemots and seals¹. In addition to the PCB metabolites—hydroxychlorobiphenyls—we unexpectedly found two compounds which after treatment with diazomethane gave mass spectra consistent with *p,p'*-DDE (1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene) containing a methoxy group ($M=346$ a.m.u.). To verify these observations rats were given *p,p'*-DDE (about 20 mg per animal dissolved in peanut oil) and the faeces investigated for the presence of phenolic metabolites as described previously¹. This report deals with the structure of three phenolic metabolites—compounds Ia–IIIa (Fig. 1)—of *p,p'*-DDE excreted by the rat.

Rats were found to excrete the same compounds as seals

Table 1 Chromatographic and mass spectral data of the methyl ethers of three metabolites of *p,p'*-DDE in the rat

Metabolite (methyl ether)	Retention time* (min)	R_f value on TLC†	346(M)	331	Mass spectrum‡ (—a.m.u.)	311	303	296	276	233
Ib	11.4	0.63	100	5	2	—	86	11	48	
IIb	13.1	0.28	100	—	9	—	8	73	43	
			(100)	—	9	—	9	65	26)	
IIIb	14.8	0.22	100	5	12	2	9	73	38	
			(100)	9	10	2	8	47	40)	

*GLC was performed on a Varian 1400 instrument with an electron capture detector (³H). Glass columns (520×0.2 cm) containing 2% Apiezon L (purified⁴) on Chromosorb W (100–120 mesh, AW, DMCS), at a column temperature of 245 °C were used. Gas flow (N₂) was about 30 ml min⁻¹.

† R_f values are given relative to *p,p'*-DDE ($R_f=1.00$) and were determined on silica gel plates (0.25 mm, Kieselgel 60, F₂₅₄, Merck) which were developed with hexane.

‡Mass spectra were obtained at 70 eV on a Hewlett-Packard 5930A instrument with a 5933A computer on-line. Calculations are based on ions containing ³⁵Cl only. Values of the synthetic compounds are given in parentheses.

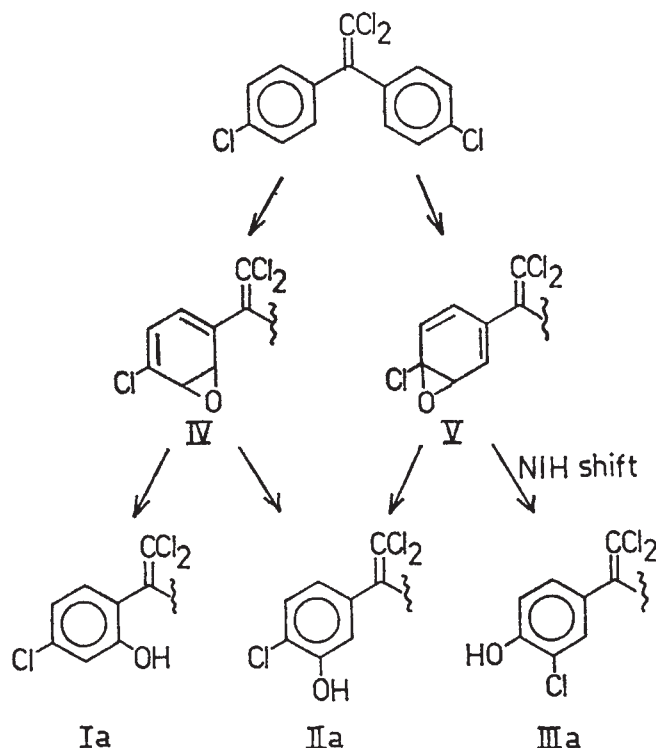


Fig. 1 Suggested metabolic route for *p,p'*-DDE in rats.

and guillemots and the structure of these (compounds IIa and IIIa, Fig. 1) have now been established by synthesis and subsequent comparisons using gas-liquid (GLC) and thin layer (TLC) chromatography and mass spectrometry (MS) of their methyl ethers (IIb and IIIb, Table 1). In addition, rats excreted a third compound which after a similar methylation gave a molecular ion at 346 a.m.u. in the mass spectrum. This compound has been given the structure Ib (Table 1) on the basis of data from chromatography and mass spectrometry. The total amount of phenolic metabolites, IIa being the major one, excreted during 6 d after dosing was estimated to be about 5% of the dose.

Methyl ethers IIb and IIIb were synthesised by the routes given schematically in Fig. 2 (G. S., unpublished). The synthetic products gave the same retention times on several GLC columns as the methyl ethers of the metabolites isolated from the rats. Comparisons of mass spectra showed only small differences in two of the peaks found useful for the identification of this type of compounds, that is, at 276 and 233 a.m.u. (Table 1). It cannot be excluded, however, that the differences found are caused by impurities from the extracted biological material.

Compound Ib, derived from the phenol Ia, had a relatively short retention time on GLC which indicated the presence of a substituent in a 2 position on the phenyl rings. This effect is well known in the case of DDT and derivatives (DDD, DDE and so on), the *o,p'* isomers eluting faster than the *p,p'* isomers². Similarly, introduction of methoxy groups or chlorine atoms in the 2 position of biphenyl give considerably shorter retention times than the 3 and 4-substituted isomers³⁻⁵. Compound Ib also had a larger R_f value on TLC than isomers IIb and IIIb, and similar relationships were found in the case of isomeric methoxychlorobiphenyls³.

Further proof for the structure Ib was given by the mass spectrum, which in contrast to those of IIb and IIIb shows a large fragment at $M-\text{CH}_3\text{Cl}$ (Table 1). This fragmentation pattern has been found in similar compounds, for example, 2-methoxychlorobiphenyls³ and 2-methoxychlorodiphenyl

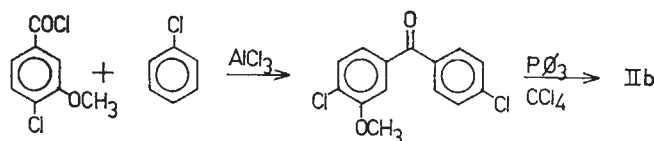


Fig. 2 Synthetic route (simplified) used for the synthesis of compounds IIb and IIIb.

ethers^{6,7}, which through the loss of CH_3Cl give rise to stable cyclic structures.

Only two of the described metabolites—Ia and IIa—are expected to be formed by a simple metabolic pathway (Fig. 1). Obviously the metabolite IIIa is formed by an NIH shift⁸ of the chlorine atom which results in a rearranged product. This phenomenon, originally observed with heavy hydrogen isotopes, has been described previously in the case of other aromatic halogen compounds⁹ and has been regarded as proof of the involvement of arene oxides (for example, IV and V, Fig. 1) in the metabolic process. The carcinogenicity of certain polycyclic hydrocarbons has been attributed to the formation of such arene oxides by enzymatic action⁸.

p,p'-DDE has hitherto been considered as a very stable metabolite of *p,p'*-DDT (1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane) and the only breakdown product proved so far is DBP (*p,p'*-dichlorobenzophenone); but DDA (2,2-bis(4-chlorophenyl) acetic acid) has also been suggested (for a recent review, see ref. 2). It is remarkable that these phenolic metabolites of this otherwise extensively studied compound have not been detected previously. Hydroxylated metabolites of the DDT group of compounds have been described only for the *o,p'* isomer of DDT (ref. 9) in which one of the *para* positions of the aromatic rings is unsubstituted.

Our results with *p,p'*-DDE, together with the recently reported metabolic hydroxylation of highly chlorinated biphenyls (6 Cl) by rats¹⁰, mice¹⁰ and rabbits¹¹, clearly indicate that even highly chlorinated compounds are slowly converted by mammals. It has, however, been shown that the highly fat-soluble phenols thereby released are more toxic than the parent compounds¹². Further knowledge of the fate of these phenols seems, therefore, to be of great importance.

This work was supported by the Research Committee at the National Swedish Environment Protection Board.

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Mating-induced behavioural switch in female fireflies

BARBER¹ reported that courting male fireflies of the genus *Photinus* receive answers from *Photuris* females, and observed *Photuris* females devouring *Photinus* males. Lloyd has summarised many of these observations and he coined the term firefly *femmes fatales* to describe *Photuris* females that mimic the responses of other species²⁻⁴. We have found that non-mated *Photuris versicolor* females answer their homospecific male flashes almost exclusively. After mating they become aggressive mimics which respond to the flashes of *Photinus macdermotti* males.

Photuris firefly larvae collected in autumn were cultured using the techniques of McLean et al.⁵. The following July seven virgin females were placed in individual terraria in a field populated by large numbers of *Photuris versicolor* and *Photinus macdermotti* males. Each evening these females were presented with the triple flash typical of males of their species and with pairs of flashes similar to those of male *Photinus macdermotti*. Flashes, simulated with manually operated incandescent bulbs⁶, varied as shown in Table 1. Intervals between presentation of flashes varied but were less than 10 s. The timing of the artificial male flashes and the female answering flashes was recorded on a tape recorder and later transferred to a polygraph. Three virgin females were mated after each had been placed on one side of a clear plastic container with a clear, removable divider separating it from a male. After the pair had exchanged signals the divider was removed and the animals converged and copulated after approximately 30 min. After the animals had separated, the female was returned to her

Table 1 Response of *Photuris* females to homospecific and heterospecific artificial flashes

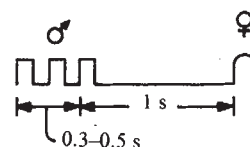
Female no.	Mated or non-mated	Heterospecific		Homospecific	
		Male (RR)	Flashes (N)	Male (RR)	Flashes (N)
1	Non-mated	0	205	0.599	519
2	Non-mated	0	166	0.544	349
3	Non-mated	0.064	233	0.578	552
4	Non-mated	0.256	246	0.571	478
5	Non-mated	0.235	51	0.443	115
	Mated	0.479	512	0	207
6	Non-mated	0	58	0.479	73
	Mated	0.454	130	0	123
7	Non-mated	0	53	0.491	106
	Mated	0.505	202	0	158
8	Unknown	0	166	0.544	349
9	Unknown	0.480	329	0	202

RR, response ratio; N, number of tests.

Heterospecific male flashes—a pair of flashes similar to those produced by a *Photinus macdermotti* male⁸ and the female answer timed as follows:



Homospecific male flashes: species-specific, triple pulse flashes characteristic of a *Photuris versicolor* male^{1,9} and the female answer timed as follows:



Fireflies were classified on the basis of the pattern of male flashes¹.

terrarium and retested on subsequent evenings. Two more females were caught in the field, placed in individual terraria and tested. The responses of all females to the homospecific male flash and heterospecific flash of *Photinus macdermotti* males are shown in Table 1. Female response declined with continual testing. Furthermore, after long periods of isolation or sudden changes of flash parameters females answered inappropriate flashes. These answers were, however, at a significantly longer latency than those to homospecific flashes, and were considered to be independent of the flash parameters and caused by the female's previous response history. They were, therefore, not counted.

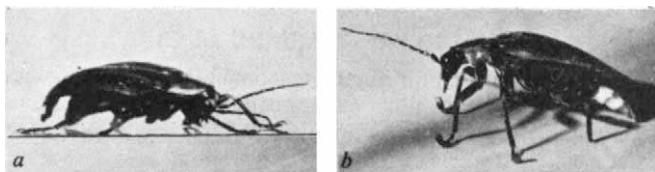


Fig. 1 *a*, Non-mated *Photuris* female answering posture. Note the hunched position and depressed antennae ($\times 2.3$). *b*, Mated *Photuris* female answering posture. Note the erect posture, extended mandibles and elevated antennae. The female has twisted her lantern toward the stimulus flash ($\times 2.3$).

Virgin females usually answered homospecific flashes, and occasionally heterospecific flashes (females 3 and 4, Table 1) but with a more variable latency and a noticeably weaker flash, responding only when the lantern's sixth segment was flashing at low intensity. No virgin female failed to respond to the homospecific flash below a response ratio of 40% (Table 1), even if she had been in captivity for more than a month.

In courtship the virgin female adopted a characteristic posture (Fig. 1*a*). When answering the male she stood hunched close to the substrate, and made a flash gesture, turning her abdomen to the side so that her lantern pointed towards him. After copulation the female's lantern glowed or she flashed spontaneously. She no longer responded to the species-specific signal and did not eat her mate. During the 2 d immediately after mating the female did not respond to any flashes.

Within three nights after mating, however, the female's behaviour changed. She now answered the heterospecific flash of *Photinus macdermotti* (females 5, 6 and 7, Table 1). Furthermore, she now adopted a characteristic erect and alert posture, standing with legs extended, antennae up and mandibles out (Fig. 1*b*). She moved actively about and her prey capture behaviour was apparently visually steered. She was now a *femme fatale*.

Two females of unknown mated state were placed in adjacent terraria and tested with homospecific and heterospecific flashes simultaneously. Female 8, who exhibited virgin posture, answered 53.7% of the homospecific flashes ($n=54$) and no heterospecific flashes ($n=51$). Female 9, a postural *femme fatale*, answered 55.2% of the heterospecific flashes and no homospecific flashes. (Table 1 shows the total number of responses of these females to all tests.) Thus two behaviours, flash communication and posture, vary in a predictable way and may be used in assessing the mating history of the female.

On the basis of these observations several of Lloyd's questions can be answered. A *Photuris* female only becomes a *femme fatale* after having mated. When a virgin female attracts her homospecific male she is not predaceous, and when the mated female is predaceous she no longer answers her homospecific male's triple flash. Thus, the *Photuris* male avoids the fate of the attracted *Photinus* male. Changes in behaviour induced by mating have been reported in female ceropia moths, and neurosecretory

activity has been implicated⁷. Implantation of the bursa copulatrix or injection of haemolymph from mated into virgin moths induced behavioural changes. The *Photuris* female may respond in similar fashion and thereby provide a simple behavioural assay system for comparable neurosecretory changes. Mating seems to induce several modifications of central nervous activity in the *Photuris* female because it changes her locomotor activity, answering posture, predatory behaviour and response to flash signals.

We thank Miss P. Nye for allowing us to study fireflies on her property, J. Buck, R. Hoy and J. Lloyd for criticism of the manuscript and A. Moiseff for assistance. This work was supported in part by a grant to the State University of New York, Stony Brook, from the US National Science Foundation.

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5-Hydroxymethyl-2'-deoxyuridine, a normal DNA constituent in certain *Bacillus subtilis* phages is cytostatic for mammalian cells

WE have described¹ the discovery of cytostatic action on cultured Ehrlich ascites carcinoma cells of a series of deoxyridines substituted in the carbon-5-position (for example, 5-hydroxymethyl-, 5-mercaptopmethyl-, 5-cyano-, 5-thiocyanato- and 5-vinyl-2'-deoxyuridine), the strongest effect being shown by 5-hydroxymethyl-2'-deoxyuridine (HMUdR). Although Green *et al.* reported² that the compound inhibits growth and cell division of *Escherichia coli* 15T-, no reports of any inhibitory properties have to our knowledge appeared since 1957. It was found, however, that HMUdR is a normal constituent of the DNA of certain *Bacillus subtilis* phages³⁻⁵, in which it replaces all the thymidine, and a number of reports have described its chemical synthesis⁶⁻¹¹. We indicate here, however, that the strong effect of the compound on Ehrlich ascites carcinoma cells is not the result of an anomalously high sensitivity of these cells in the culture conditions used, but is a more general phenomenon also found with other cell lines and *in vivo*.

HMUdR was prepared from 3', 5'-di-O-acetylthymidine by selective monobromination of the carbon-5-methyl group by bromine in tetrachloromethane under ultraviolet catalysis and hydrolysis of the resulting 5-bromomethyl-2'-deoxy-3', 5'-di-O-acetyluridine by a saturated NaHCO₃ solution. Deacetylation with methanolic ammonia gives the desired compound (80-90% overall yield). Elemental analysis was correct, and melting point (180-181 °C) and proton magnetic resonance spectra compared well with those reported by other authors^{10,11}.

Table 1 shows that HMUdR has a strong cytostatic action on most cell lines. The only exception are HeLa cells, which for some unknown reason are hardly influenced by the compound. As has been shown earlier with Ehrlich ascites carcinoma cells, the inhibition can be prevented by thymidine at a concentration of about one-tenth of that of the inhibitor¹. After 24 h, however,

Table 1 Inhibition of proliferation of different cell lines by 5-hydroxymethyl-2'-deoxyuridine

Cell type	Inhibition (%)			
	1×10^{-6}	1×10^{-5}	5×10^{-5}	1×10^{-4}
Vero	0	80	100	100
L 929	ND	0	ND	80
HeLa	ND	0	ND	0
HeLa (S)	0	0	ND	0
BHK 21/C13	ND	100	ND	ND
BHK 21/C13 (S)	80	100	ND	100
HEF	ND	60	ND	100

A substrain of BHK 21/C13 cells (strain P-2, originally obtained from P. B. Capstick¹²) and HeLa cells were maintained in suspension culture as described elsewhere¹³. For the experiments reported here these cells were also used as monolayers as were L929 mouse cells, Vero cells, and a human embryonic fibroblast line (HEF). Cells from the stationary phase of growth were resuspended in fresh growth medium (Eagle's MEM supplemented with 10% tryptose phosphate broth, 10% foetal calf serum, and neomycin) containing the inhibitor at the concentrations indicated, and seeded into test tubes or suspension vessels, respectively. The influence of HMUdR on cell proliferation was followed over a period of 48–72 h, when the control cultures of the different cell lines tested had performed two to three rounds of replication. Each experiment was carried out at least in duplicate. Cells were counted in a Fuchs–Rosenthal chamber (monolayer cells after trypsinisation).

S, Cells in suspension culture.

ND, Not determined.

the inhibition was irreversible. Note that thymidine itself is inhibitory at very high concentrations only, for example, 1×10^{-2} M for BHK cells.

A cytostatic action could also be shown in *in vivo* experiments with mice. A dose of 120 mg kg⁻¹ given every 8 h for 3 successive days was lethal. The mice died of diarrhoea and leukopenia (that is, typical toxic effects of cytostatic drugs). Experiments are under way to check whether with reduced doses and modified dose schedules the compound may become a useful cancerostatic agent.

Preliminary evidence from experiments with Ehrlich ascites carcinoma cells on ³²P-phosphate incorporation into DNA (not immediately inhibited) and on ³H-thymidine incorporation into DNA (immediately inhibited) as well as from pulse cytophotometric studies suggests incorporation of HMUdR into DNA. Since the compound normally replaces thymidine in the DNA of *B. subtilis* phages, this incorporation *per se* cannot affect basic functions of the DNA helix. It may, however, interfere with some more specific DNA–protein interactions (as in the attachment of DNA to membranes or within the eukaryotic chromatin). Glucosylation, as was found for the majority of 5-hydroxymethyl-2'-deoxycytidine residues in the DNA of the T even phages of *E. coli*^{14,15} (but not for the HMUdR residues in the DNA of the above mentioned *B. subtilis* phages), may serve to prevent the adverse effects of a hydroxymethyl side chain at the carbon-5 of DNA pyrimidines. In this context, it would be of interest to see to what extent HMUdR is inhibitory for other organisms and viruses, for example, coliphages, animal viruses and various bacteria.

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Growth of B lymphocyte colonies *in vitro* from mouse lymphoid organs

TECHNIQUES now exist for obtaining clonal growth in semi-solid agar of mouse neutrophil, macrophage, eosinophil, erythropoietic and megakaryocytic colonies^{1–5}. Previous attempts to obtain colony formation by mouse lymphoid cells, however, were unsuccessful and cultured lymphocytes died very quickly, even in the presence of known lymphocyte mitogens. Mercaptoethanol and related substances have been shown to increase lymphocyte proliferation in liquid cultures⁶ and colony formation in agar by mouse plasmacytoma cells⁷. Based on these observations, attempts were made to obtain clonal growth of mouse lymphoid cells in agar medium supplemented with 2-mercaptoethanol.

Dispersed cell suspensions in Eisen's balanced salt solution were prepared from the mesenteric and subcutaneous lymph nodes, Peyer's patches, spleen, thymus and bone marrow of 2–3-month-old C57BL mice. Cell suspensions were added to an equal volume mixture of double strength modified Eagle's medium⁸ and 0.6% agar, the mixture containing a final concentration of 15% unheated foetal calf serum and 5×10^{-5} M 2-mercaptoethanol. Volumes (1 ml) of the cell suspensions in agar medium were added to 35 mm plastic dishes, allowed to gel and incubated at 37 °C in a fully humidified atmosphere of 10% CO₂ in air.

In cultures of lymph node or spleen cells, extensive cell death occurred in the first 24 h but between 24 and 48 h

Table 1 Frequency of cluster- and colony-forming cells in C57BL mice

Tissue	No. of experiments	Frequency per 10 ⁶ cultured cells*	
		Clusters	Colonies
Mesenteric lymph nodes	21	4,002 ± 2,716	870 ± 355
Subcutaneous lymph nodes	7	2,621 ± 1,114	712 ± 222
Peyer's patches	5	3,299 ± 2,839	804 ± 582
Spleen	7	5,082 ± 3,220	1,167 ± 388
Thymus	10	2 ± 2	1 ± 1
Bone marrow	5	2,656 ± 474	444 ± 229

*Pooled organs from two to five C57BL mice aged 2–3 months used in each experiment (1×10^6 cells cultured per dish). All cultures contained 1 ml Dulbecco's modified Eagle's medium⁸ in 0.3% agar with a final concentration of 15% foetal calf serum and 5×10^{-5} M 2-mercaptoethanol. Cluster counts (2–50 cells) performed on day 4; colony counts (> 50 cells) on day 7. Mean data from four replicate cultures ± s.d.

surviving cells began to proliferate and by day 4 had generated large numbers of discrete clusters of cells containing 2–50 cells. On continued incubation to day 7, many clusters increased in size to form colonies of 50–800 cells (Fig. 1). In some cultures, colony growth continued beyond day 7 but usually colony and cluster cells died between day 8 and 14 of incubation. Microscopic examination of cluster and colony cells between days 2 and 5 showed the cells to be a uniform population of large mononuclear cells with a round nucleus and bulky basophilic cytoplasm, similar in general appearance to lymphoid cells stimulated in liquid cultures by phytohaemagglutinin or pokeweed mitogen. By days 7 to 10, many colony cells were of

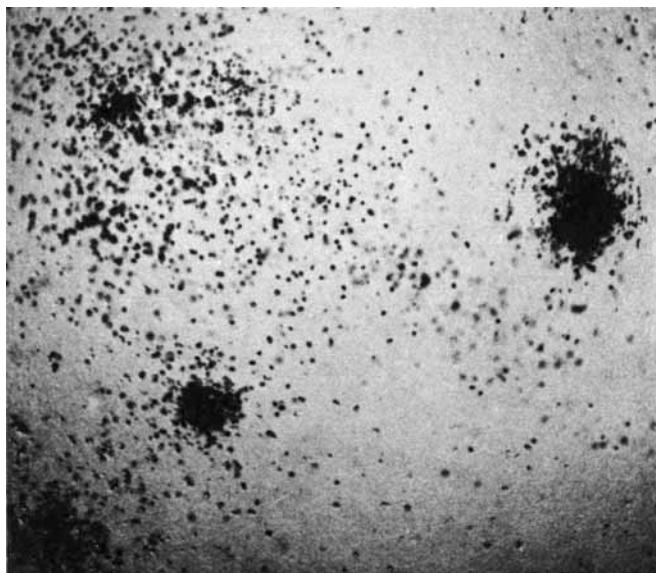


Fig. 1 Colonies (7 d) grown in agar medium from C57BL mesenteric lymph node cells.

smaller size and some resembled immature plasma cells with an eccentric condensed nucleus, basophilic cytoplasm and a prominent Golgi region. Less frequently, cells resembled small or medium lymphocytes although classical small lymphocytes were not seen. Mitotic activity was high in 2–4-d clusters and colonies (3–4%) but fell to below 1% in 7–10-d colony cells.

As shown in Table 1, clusters and colonies regularly grew in cultures of 1×10^6 C57BL mesenteric and subcutaneous nodes, Peyer's patches, spleen and marrow, but not in thymus cultures. Analysis of the bone marrow cultures indicated that most of the clusters and colonies were composed of neutrophils and macrophages. Clusters and colonies of this type were only occasionally seen in spleen cultures and never in cultures of the other cell suspensions. Omission of the 2-mercaptoethanol from cultures of lymph nodes, Peyer's patches or spleen cells completely suppressed cluster and colony formation, but in the

case of the marrow cultures some cluster and colony formation was still observed. Cultures of mesenteric or subcutaneous lymph node cells from congenitally athymic (*nu/nu*) mice, grossly deficient in functional T cells, produced equivalent numbers of clusters and colonies to those produced by C57BL cells.

The incidence figures for colony- and cluster-forming cells in lymphoid cell suspensions are probably underestimates. Colony and cluster formation was slightly nonlinear in relation to the number of cells cultured at a concentration range of 6.25×10^4 ml^{-1} to 1×10^6 ml^{-1} with highest frequencies of colonies and clusters being observed with 1×10^6 cells ml^{-1} . Furthermore, cluster numbers increased progressively with continued incubation but because of dispersion of cluster cells and cell crowding, accurate cluster counts could not be performed after day 4.

To identify the nature of the cell type growing in colonies, cells from individual or pooled colonies from 4–7-d cultures of C57BL mesenteric node were harvested, washed free of agar and tested for the presence of Fc or C3 receptors or membrane-bound immunoglobulin. Fc receptors were detected by rosette formation with sheep red cells coated with rabbit or mouse IgG anti-erythrocyte antibody or by sequential incubation with high molecular weight ($>15 \times 10^6$) aggregated human γ globulin (HGG) and fluorescein-conjugated rabbit anti-HGG. C3 receptors were detected by rosette formation with erythrocytes coated with antibody complement, and membrane Ig by incubation of cells with fluorescein-conjugated goat anti-mouse μ chain (Meloy, Virginia) or polyvalent rabbit anti-mouse Ig serum. The results (Table 2) indicate that 23–39% of colony cells exhibited Fc receptors, all were negative for EAC, and 50–76% of mass-harvested colony cells were Ig-positive. Micromanipulated individual colony cells, which possess the advantage of being entirely free of adherent agar, were positive for cell membrane immunoglobulin in 96% of cases using cells collected between days 2 and 9 of culture. The median intensity of staining was less than that of spleen B lymphocytes. Capping was observed in 70% of colony cells when incubated in the presence of these antisera at 37 °C. Pinocytosis of fluorescein-conjugated anti- μ seemed to be particularly rapid. Control bone marrow neutrophilic, macrophage and eosinophilic colony cells collected from agar cultures in similar conditions were uniformly negative for surface immunoglobulin even when the marrow cultures had been grown in cultures containing 1×10^6 lymph node cells.

On the basis of the above characteristics, the cells in the clusters and colonies developing in cultures of mouse lymphoid organs have been identified as belonging to the B lymphocyte series. Further experiments are required to demonstrate formally that the surface immunoglobulin represents synthesised immunoglobulin but the present culture system should be a valuable new technique for analysing normal or antigen-stimulated lymphoid populations.

This work was supported by the Anti-Cancer Council of Victoria; the National Cancer Institute, Washington and the Swiss National Foundation for Scientific Research.

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Table 2 Surface markers of colony and cluster cells grown from C57BL lymph nodes and spleen

Type of colony cells	Assay	No. of positive cells No. tested
Pooled 4-d mesenteric lymph node colonies	EA rosettes	57/243 (23%)
Pooled 4-d spleen colonies	EAC rosettes	5/379 (1%)
Pooled 6-d NZB mesenteric lymph node colonies	Aggregated HGG receptor	21/84 (25%)
Pooled 4-d mesenteric lymph node colonies	Aggregated HGG receptor	32/108 (30%)
Pooled 6-d mesenteric lymph node colonies	Aggregated HGG receptor	12/31 (39%)
Pooled 4-d mesenteric lymph node colonies	Fluorescein-conjugated anti- μ	84/151 (56%)
Pooled 6-d mesenteric lymph node colonies	Fluorescein-conjugated anti- μ	76/100 (76%)
Pooled 7-d Peyer's patch colonies	Fluorescein-conjugated anti- μ	11/22 (50%)
Pooled 7-d subcutaneous lymph node colonies	Fluorescein-conjugated anti- μ	10/15 (66%)
Individual 2–9-d mesenteric node colony cells	Fluorescein-conjugated anti- μ	99/191 (52%)
		68/71 (96%)

Positive controls for Fc receptor assays were 7 d macrophage and neutrophil colony cells grown from bone marrow cells (175/304 positive), peritoneal macrophages (75/99 positive) and S49 lymphoma cells (114/116 positive). Negative controls were thymus cells (0/100 positive); W232 lymphoma (T-cell) (2/300 positive). Positive controls for anti- μ were mouse spleen cells (38/97 positive), negative controls were thymus cells (2/372 positive) and 7 d pooled macrophage and neutrophil colonies grown from marrow cells with or without added lymph node cells (2/443 positive). Micromanipulation of agar colony cells and their staining in fluorochromes was modification of standard de Fonbrune techniques.

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Inhibitory effect of external sodium ions on the potassium pump in striated muscle

Frog muscles with elevated sodium and depleted potassium contents recover to their normal low sodium and high potassium state in the presence of external K^+ ions^{1,2}. The recovery process involves active Na extrusion, an electrogenic hyperpolarising action of the Na pump and an active inward transport of K^+ ions^{3,4}. Frog striated muscle fibres, in common with other cell types, show an increased rate of sodium extrusion when external Na^+ ions are removed and are replaced with an inert substance such as Tris or choline⁵⁻⁷.

As a part of the outward active transport of Na is widely believed to be coupled to the active inward transport of K, it was of interest to see if Na-free conditions in the external medium have the same effect on K influx as on Na efflux in muscle cells.

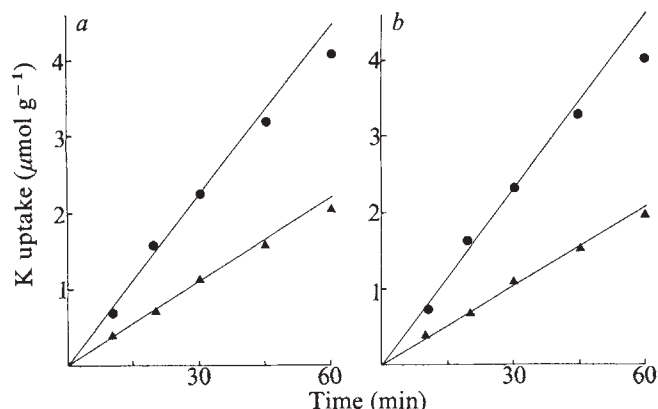


Fig. 1 Potassium uptake measured with ^{42}K ions when $[K]_0 = 0.5$ mM is plotted against time. *a*, The results were obtained on paired muscles. Uptake was measured in Na-Ringer solution on one muscle and in Na-free, Tris-substituted Ringer solution on the other muscle of the pair. ●, 0.5 mM K Tris; ▲, 0.5 mM K, 105 mM Na. *b*, Measurements were made on paired muscles in Na-free media. One muscle was in the presence of 10^{-5} M strophanthidin, the other muscle serving as a control. ●, 0.5 mM K Tris; ▲, 0.5 mM K Tris, strophanthidin.

Frog sartorius muscles were enriched with Na^+ ions by immersion in K-free Ringer solution at 4 °C for 18 h. Potassium influx in such muscles was then measured by the ^{42}K technique in both normal Ringer solution and in Na-free Ringer solution with Na replaced by Tris. Figure 1*a* shows a typical experiment in which a large increase in K^+ influx occurs when external Na is replaced by Tris. Figure 1*b* shows that this increase in K^+ influx is abolished by application of 10^{-5} M strophanthidin. Similar experiments were performed at a number of values of $[K]_0$. The strophanthidin-sensitive fraction of the measured K

Table 1 Membrane potentials in the presence and absence of external Na

$[K]_0$ (mmol l ⁻¹)	$[Na]_0$	$E_m \pm s.e.$ no. of observations (mV)	E_K^*
0.5	105	-108.0 ± 2.4 (5)	-130.6
0.5	0	-106.0 ± 1.4 (6)	-130.6
1.0	105	-102.9 ± 2.1 (7)	-113.1
1.0	0	-123.8 ± 1.2 (6)	-113.1

* Calculated at 20 °C from values of $[K]_0$ used and values of $[K]_i$ determined by flame photometry.

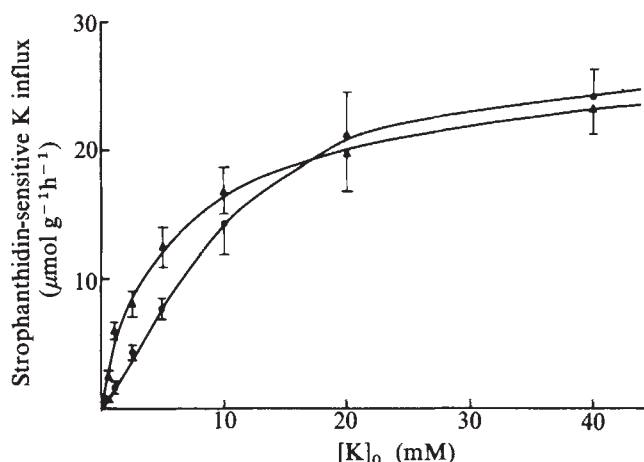


Fig. 2 The strophanthidin-sensitive component of K influx measured with ^{42}K ions using Na-enriched muscles is plotted against the potassium ion concentration in the medium, $[K]_0$. Each point represents the average value obtained using six or more measurements. Vertical bars represent ± 1 s.e. ●, measurements made in Na-Ringer solution (105 mM Na); ▲, measurements made in Na-free, Tris media.

influx is plotted against $[K]_0$ in Fig. 2 both in the presence and absence of external Na. The results show that external Na ions have a significant inhibitory effect on the strophanthidin-sensitive K influx at all values of $[K]_0$ below 10 mM. The effect is most pronounced at low values of $[K]_0$.

Some of this effect could be due to an increased electrogenic action of the Na pump under Na-free conditions. This action could result in an increased passive influx of K. To study the possible influence of electrogenic action of the Na pump, membrane potentials were measured in the same conditions at two values of $[K]_0$ where a large increase in K influx occurs on removal of external Na. The results are presented in Table 1.

Table 2 Changes in K content in the presence and absence of external Na

$[Na]_0$ (mmol l ⁻¹)	Initial K content	Final K content	Change in K content
105	56.2 ± 1.8 (8)*	52.6 ± 1.6 (8)	-3.6 ± 1.9
0	55.0 ± 1.7 (9)	63.5 ± 1.9 (9)	$+8.5 \pm 1.9$

Sodium-enriched muscles were placed in Na and Na-free Ringer solution with $[K]_0 = 0.5$ mM for 2.5 h and were then analysed for K content by flame photometry. The initial K contents were obtained by analysing one member of a pair of muscles from the same frog for K at the time the other member of the pair was placed in Ringer solution containing 0.5 mM K.

* $\pm$ s.e. (n observations)

Included in the table are calculated values of the K^+ equilibrium potential using the average value of $[K]_i$ measured by flame photometry. Table 1 shows that when $[K]_0 = 0.5$ mM, potentials measured in the presence or absence of $[Na]_0$ are of comparable magnitude and are less negative than E_K . A passive net loss of K from the fibres is thus predicted in these conditions. The increased K influx occurring when external Na is removed cannot, therefore, be the result of an enhancement of the electrogenic potential created by the Na pump. When $[K]_0 = 1.0$ mM, however, the situation is quite different. A large hyperpolarisation occurs when external Na is removed and potentials in the absence of Na are more negative than E_K . The hyperpolarisation occurring when Na is removed was abolished by 10^{-4} M ouabain. When $[K]_0 = 1.0$ mM, the increased K influx could have occurred because of the enhanced electrogenicity of the Na pump.

As a further test for an inhibitory effect of external Na on

the K pump, net changes in K content were measured over a 2.5-h interval by flame photometry when $[K]_0 = 0.5$ mM. The results are shown in Table 2. Muscles in Na Ringer solution lost K whereas muscles in Na-free Ringer solution gained K against an electrochemical gradient.

The flux and membrane potential data lead to the conclusion that external Na^+ ions have an inhibitory effect on the K^+ ion pump in muscle fibres when $[K]_0 = 0.5$ mM. The effect probably exists at other values of $[K]_0$ but this cannot be concluded with certainty from the present data. This work was supported by a grant from the National Institute of Neurological Diseases and Stroke.

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Received April 7; accepted April 15, 1975.

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Quantitative increase of neuroglia-specific GFA protein in rat C-6 glioma cells *in vitro*

AMONG neural cell lines the C-6 glioma, a cell line cloned from an N-methylnitrosourea (MNU)-induced experimental glioma in rat, has generated considerable interest as a model system for the study of neuroglia¹. The clone was originally selected on the basis of a high production of the protein S-100, a protein believed to be characteristic of glial cells *in vivo*². Since its introduction, the line has been further characterised with regard to a number of other biochemical features³. Among these is the recent demonstration of the glial fibrillary acidic

(GFA) protein by immunofluorescent staining of C-6 cells maintained in certain *in vitro* conditions⁴. The GFA protein, isolated from multiple sclerosis plaques and areas of subependymal gliosis in the central nervous system, has been shown to be a specific marker for fibrillary astrocytes, is believed to be a component of the 80–90 Å diameter glial filaments^{5–8}, and has not been reported in non-glial neural cell lines. It is, therefore, a useful biochemical marker for the differentiation of a type of mature astrocyte. In previous work with the C-6 line, GFA protein was found to be present by immunofluorescence in only an occasional C-6 cell maintained in monolayer or suspension cultures, and to be markedly increased in many C-6 cells when they were maintained for 12–14 d in an organ culture system using Gelfoam matrices⁴. Such a system is known to favour structural differentiation *in vitro*⁹. No increase of filaments was observed, however, in any of the *in vitro* systems used, suggesting that the synthesis of GFA protein could proceed without the formation of intracytoplasmic glial filaments⁴. We compared the quantitative levels of GFA protein in C-6 and other cell types in various culture conditions, using a “two-site” immunoradiometric assay recently developed in this laboratory¹⁰.

C-6 cells and mouse strain L-929 fibroblasts, both obtained from the American Type Culture Collection, were maintained at 36–37 °C in several systems. Monolayer cultures were grown in Roux bottles in a medium consisting of minimal essential medium—Spinner solution (Grand Island Biological Co., Grand Island, New York), sodium bicarbonate, supplemental glutamine (0.3 mg ml⁻¹), and 10% immunoprecipitin-tested foetal calf serum⁴ (v/v). Penicillin (50 U ml⁻¹) and achromycin (20 µg ml⁻¹) were also added. Parallel monolayers were grown in enriched medium^{4,11} consisting of unfiltered foetal calf serum (40 ml) (Reheis Chemical Co., Chicago); Simms' balanced salt solution (35 ml); bovine serum ultrafiltrate (25 ml) (Microbiological Association Inc., Albany, California); concentrated dextrose solution (3 ml) (final concentration in the complete medium, 6 mg ml⁻¹); insulin (3 ml) (starting concentration, 3 U ml⁻¹; final concentration, 0.09 U ml⁻¹) (low zinc insulin, courtesy of Squibb Institute for Medical Research, New Brunswick, New Jersey); stock penicillin (1 ml) (final concentration, 50 U ml⁻¹) and stock

Table 1 GFA protein levels in cells, media and matrices

	GFA protein† (ng per assay tube, 100λ)		GFA protein (ng per µg DNA)		
C-6 cells					
Monolayer (Spinner)*	3.80	3.41	0.07	0.03	(0.05)‡
Monolayer (enriched)	0.17	0.24	0.28	0.35	(0.32)
Suspension (Spinner)	0.12	0.23	0.03	0.09	0.08 (0.07)
Organ culture (Spinner)	0.68	6.60	0.68	1.64	(1.16)
Organ culture (enriched, 6 d)	11.90	1.01	2.90	1.68	(2.29)
Organ culture (enriched, 13 d)	2.80	7.80	2.07	1.95	(2.01)
L cells					
Monolayer (Spinner)	0.53	0.12	0.01	0.01	(0.01)
Monolayer (enriched)	0.13	0.08	0.03	0.01	(0.02)
Suspension (Spinner)	0.06	0.13	0.01	0.02	0.01 (0.01)
Organ culture (Spinner)	0.70	0.42	0.01	0.01	(0.01)
Organ culture (enriched)	0.31	0.12	0.02	0.04	(0.03)
KB cells					
Bulk suspension (Spinner)	0.23	0.11	0.01	0.01	(0.01)
Media			(ng per ml media)		
Earle's BSS (1X)	0, 0, 0, 0.20		0, 0, 0, 2		(0.50)
Spinner medium	0, 0, 0.20		0, 0, 2		(0.70)
Enriched (unspent)	0, 0, 0, 0, 0.20, 0.30		0, 0, 0, 0, 2, 3		(0.83)
Enriched (spent)					
Filtered	0, 0.42, 0.30, 0.13, 0.13		0, 4.2, 3, 1.3, 1.3		(1.96)
Unfiltered	0.15, 0.30		1.5, 3		(2.25)
Matrices					
Triplicate samples of Gelfoam and Spongostan were negative for DNA and GFA protein.					

* Spinner and enriched refer to media.

† Our standard curve for assay detection ranges from 0.22 to 220 ng (midpoint: 9 ng). On the basis of triplicate analyses within this range, the minimal detectable dose for GFA protein is 0.064 ± 0.022 ng.

‡ Values in parentheses indicate arithmetic means.

achromycin (1 ml) (final concentration, $20 \mu\text{g ml}^{-1}$). Both cell types were also maintained in rotatory suspension cultures in Spinner medium¹² and pellets from these cultures were explanted on to Gelfoam or Spongostan matrices in an organ culture system, as previously described¹¹. These organ cultures were grown in parallel in Spinner and enriched medium, and terminated after 6 and 13 d. At the time of termination, the cells and explants were washed once with Earle's balanced salt solution (BSS) before freezing for biochemical analysis. Parallel cultures were fixed at equivalent time intervals and their viability monitored by light microscopy⁴. Cell samples obtained in these different culture conditions were divided in half on the basis of wet weight (monolayers), cell count (suspension cultures), or number of explants (organ culture system). Half was used for the determination of total DNA content by the spectrophotometric method of Burton¹³, and half was extracted with 0.05 M phosphate buffer (pH 8) containing 0.0001 M EDTA for the assay of GFA protein levels by the two-site immunoradiometric method¹⁰. The solid explants and the underlying matrices (Gelfoam or Spongostan), which contained numerous invading cells, were submitted for analysis. Pellets of KB cells maintained in bulk suspension culture were treated identically.

The GFA protein content of the various cell types in different culture conditions is summarised in Table 1. The levels of GFA protein in C-6 cells maintained in monolayer or suspension cultures using Spinner medium are only slightly increased compared with the trace levels recorded in strain L mouse fibroblasts and KB cells maintained in comparable *in vitro* conditions. In the organ culture system using enriched medium, GFA protein is increased approximately sevenfold after 6 and 13 d *in vitro* compared with C-6 cells maintained for 13 d in monolayer cultures using the same medium. Using Spinner medium, the increase of GFA protein in C-6 cells maintained in organ culture is 23-fold. Since the use of an enriched medium compared with Spinner medium results in only a sixfold increase in the very low level of GFA protein in C-6 cells maintained in monolayer cultures, and in only a twofold increase in the much higher level of GFA protein in cells maintained in organ culture systems, it is reasonable to conclude that the organ culture system is the most important factor favouring an increase in GFA protein. Enrichment of the medium plays a lesser role. When both these factors are taken into account, there is an approximately 40-fold increase of GFA protein in C-6 cells maintained with enriched medium in organ culture when compared with the level in monolayer cultures maintained in Spinner medium.

Analysis of the media and matrices is also summarised in Table 1. GFA protein is not present in significant quantities in the media and is absent in the matrices. Immunoradiometric analysis of the spent enriched medium (after removal of cells by filtering through a $0.45 \mu\text{m}$ Millipore filter) demonstrates very low levels of GFA protein, suggesting that the protein is chiefly intracellular.

The technique of organ culture is based on the principle of culturing explants in a moist gas phase on the surface of a relatively large volume of stationary nutrient medium; the proximity of the tissue to the surface of the medium, the large ratio of medium to cells, and the presence of a large gas-nutrient medium interface presumably result in a rapid replenishment of oxygen, adequate to meet the requirements of metabolically very active tissues. On the other hand, monolayer and suspension cultures may show considerable variation in their oxygen microenvironment, depending on the amount of the overlying fluid medium¹⁴. It is uncertain at present to what extent biochemical differentiation of the C-6 glioma cell line could be related to the more favourable rates of tissue oxygenation prevailing in the organ culture system used in this study. In any event, the Gelfoam or Spongostan matrices probably exert either a chemical effect on the surface of the cells or a purely mechanical one of providing greater opportunity for increased cell-to-cell contact. Other investigators have shown

an increase in S-100 protein levels in C-6 cells after attaining confluence in monolayer, a finding which they attribute to increased cell-to-cell contact^{15,16}. Also, cultured neurones have shown increased incorporation of labelled leucine into protein when maintained in Gelfoam compared with monolayers maintained on plastic culture dishes¹⁷. Together these findings suggest that the increased cell-to-cell contact occurring in sponge foam matrices could favour, in the relatively undifferentiated C-6 glioma cell line, increased production of a protein specific for fibrillary astrocytes.

We thank Ms Mary Ann Lawrence and Ms Y-L Lee for technical assistance and Drs D. Korn and L. J. Rubinstein for helpful advice. Pellets of KB cells maintained in bulk suspension work provided by Drs D. Korn and W. D. Sedwick. This work was supported by grants from the US Public Health Service and by the Veterans Administration.

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Histamine H₂-receptors on single central neurones

THE detection of histamine H₂-receptor antagonists^{1,2} has provided fresh impetus to the investigation of central effects of histamine³⁻⁷ and its possible role as a neurotransmitter^{8,9-10}. In the hypothalamus of rat and cat microelectrophoretically applied histamine excites a large number of cells. This action, which is unique to the hypothalamus, is not selectively antagonised by the H₁-receptor antagonist mepyramine or by the H₂-receptor antagonist metiamide^{8,9}. In the cortex of the cat several H₁-antagonists were tested but no specific action could be demonstrated¹¹. We report now on a selective antagonism of metiamide against depressant actions of histamine in the cerebral cortex of cat and rat.

Experiments were carried out on eight rats anaesthetised with a mixture of urethane (400 mg kg^{-1}) and pentobarbitone (50 mg kg^{-1}) and four cats anaesthetised with pentobarbitone (35 mg kg^{-1}). Histamine, γ aminobutyric acid

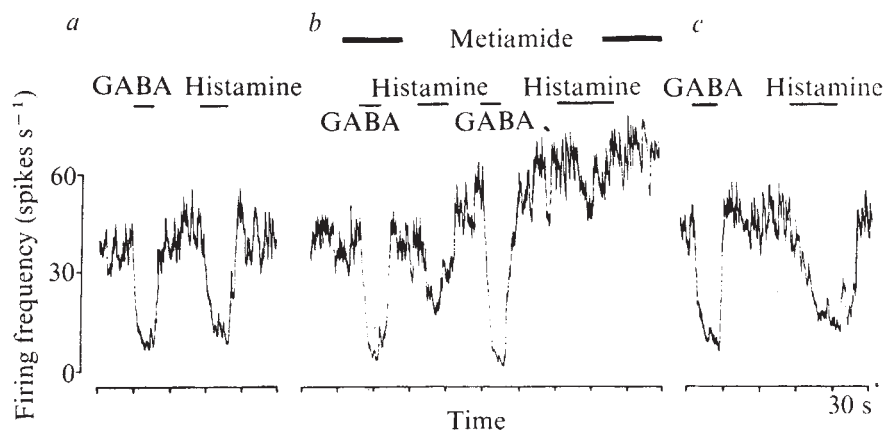


Fig. 1 Ratemeter recording showing depressant effects of GABA (0.5 M, 15 nA) and histamine (0.5 M, 50 nA) immediately before (a), during (b) and 3 min after (c) microelectrophoretic application of metiamide (kindly provided by M. Hind from SKF; 0.1 M, 30 nA) on a neurone of the rat cortex. Time of application of substances is indicated by bars.

(GABA), betazole, acetylcholine and metiamide were applied microelectrophoretically to the immediate environment of single neurones of the sensorimotor cortex using five-barrel micropipettes and conventional techniques. Histamine usually had a depressant effect on the firing of cortical neurones (21 out of 32 cells tested), which was more pronounced in the rat. Dual or excitant effects were rare (three cells), and eight cells were unresponsive to histamine. Betazole, and H_2 -agonist, had depressant actions similar to those of histamine. Metiamide reversibly reduced or blocked depressant actions of histamine on seven cortical neurones (rat: four, cat: three) and that of betazole (rat: two) without affecting the depressant action of GABA and the excitant action of acetylcholine. A depressant action of metiamide which usually occurred with higher ejecting currents (in excess of 50 nA) seemed to be unrelated to its histamine antagonism. We obtained similar results with metiamide in another series of experiments on cat mid-brain and hypothalamus. Note that on the superior cervical ganglion of the rabbit H_2 effects are depressant whereas H_1 effects are excitatory¹².

Neurochemical, pharmacological and behavioural studies suggest the implication of histamine in several central functions^{4-7,10} but in most of these studies the exact place and mode of action is unknown and a primary effect on other structures than neuronal cannot be excluded⁹. To verify these data from single neurone studies, investigations on identified systems and a specific antagonist are needed. As we found metiamide to be a reliable antagonist of depressant actions of histamine on central neurones we think that this substance may prove to be a valuable tool in further (microelectrophoretic) experiments to elucidate the role of histamine in the central nervous system.

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Potential probe for isolation of the β -adrenoceptor, chloropractolol

A TECHNIQUE which has proved highly successful in the isolation and identification of pharmacological receptors, is the use of a high affinity, selective label which binds irreversibly to the receptor¹. We report here our findings with a new irreversible, potent and cardioselective (β -1) β adrenoceptor antagonist, chloropractolol (I). This new compound is a modification (Fig. 1) of practolol (II), and represents the first known selective, irreversible β antagonist. Chloropractolol (I) was prepared with the intention of incorporating an alkylating function into the parent compound, while maintaining β -1 selectivity. An additional advantage of using practolol (II) rather than propranolol as the parent molecule, is that the resultant compound should, like practolol, lack nonspecific membrane stabilising effect² and any interaction with 5-hydroxytryptamine receptors³.

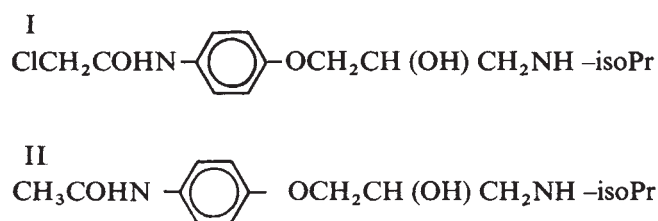


Fig. 1 Chloropractolol (I); practolol (II). Chloropractolol (I) was synthesised from 1-(4-nitrophenoxy)-3-isopropylamino-propan-2-ol⁴ by catalytic reduction of the nitro group (10% palladium on charcoal), followed by chloro-acetylation of the anilino derivative in a buffered solution (pH 6). Chloropractolol (I) was isolated and purified as its hydrochloride salt (melting point 147-148.5 °C).

Isolated rat atria were used to test the antagonistic activity of practolol (II) and its chloro analogue (I) on β -1 subtype of myocardial β adrenoceptors. Cumulative dose-response curves to isoprenaline-induced increase in chronotropic effect were obtained in the absence and presence of increasing concentrations of the antagonists as described previously⁵ (Fig. 2, for chloropractolol). The affinities of chloropractolol (I), pD_2 , and practolol (II), pA_2 , were calculated from these curves⁶ and were found to be: $pD_2 = 7.5$ and $pA_2 = 6.4$. Chloropractolol (I) is thus a significantly more potent antagonist on myocardial β -1 adrenoceptors than practolol (II).

The irreversible nature of β antagonism exhibited by chloropractolol (I) in myocardial preparations is clearly indicated by the fact that it took 3 h of repeated washing of the prep-

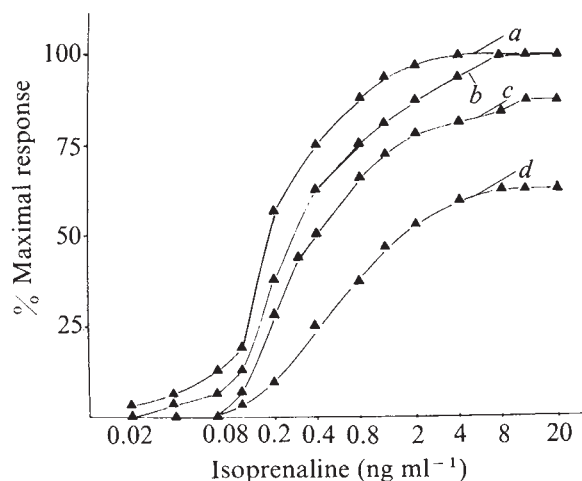


Fig. 2 Effect of three concentrations of chloropractolol (I) on the isoprenaline-induced positive chronotropic effect on rat atria. *a*, Control, isoprenaline alone; *b*, isoprenaline in the presence of 3.3×10^{-8} M chloropractolol; *c*, isoprenaline in the presence of 6.5×10^{-8} M chloropractolol; *d*, isoprenaline in the presence of 1.3×10^{-7} M chloropractolol. Data represent mean $\pm$ s.e. of responses from four atrial preparations.

aration (70 washes) until the β adrenoceptor blockade was completely reversed. In contrast, the blocking action of practolol (II) was entirely abolished within 15 min of only one wash of the atria with Krebs's solution, even though the drug was used in a concentration ten times higher than that of chloropractolol.

When tested on rat stomach fundus, a preparation which contains β -2 receptors⁵, the pD_2 of chloropractolol was 5.0, indicating that the selectivity ratio for β -adrenoceptor antagonism remained at least as high (β -1/ β -2 about 300:1) as that of practolol (β -1/ β -2 about 140:1) (ref. 5). It did not seem to damage the receptor irreversibly, as it was possible to achieve the original response to isoprenaline on the atrial preparation once the antagonist was finally removed after 3 h of repeated washings (Fig. 3). It also proved to be a specific antagonist of isoprenaline on β adrenoceptors. It did not antagonise acetylcholine or histamine-induced contractions in guinea pig ileum, in concentrations below 10^{-4} M. It is therefore suggested that chloropractolol (I) can be used with adequate radioisotope

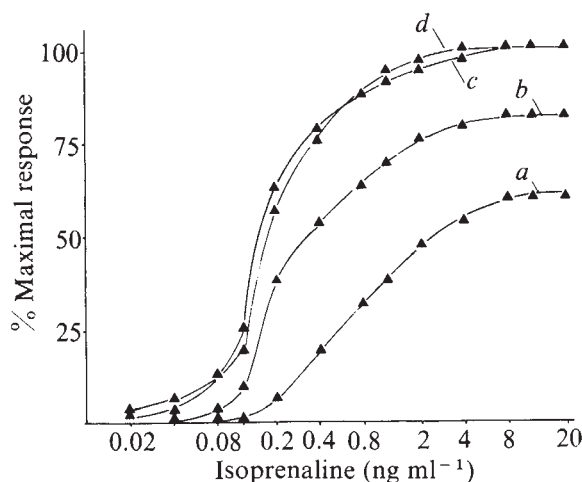


Fig. 3 Reversal of chloropractolol blockade of rat atrial response to isoprenaline, as a function of time and repeated washings. *a*, After one washing and 15 min; *b*, after 30 min and 30 washes; *c*, after 3 h and 70 washes; *d*, control. Data represent mean $\pm$ s.e. of responses from four atrial preparations.

labelling, as a tool for the identification and isolation of the β -1 subtype of adrenoceptors.

We thank Miss C. Weiss for assistance.

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Received March 25; accepted April 11, 1975.

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Binding of ovine 125 I-prolactin to cultured anterior pituitary tumour cells and normal cells

THE first report of specific, high affinity and low capacity binding of radioiodinated ovine prolactin to homogenate particles from lactating mouse mammary glands, liver and several other organs of rats and mice¹ was followed by a radioreceptor assay for prolactin using purified mammary fractions². Other reports of prolactin binding have been with reference to a variety of crude or partially purified membrane fractions³⁻⁷ or tissue slices⁸⁻¹⁰. These can be assumed to describe various degrees of submaximal binding response because of proteolysis, denaturation or structural factors that restrict free access of the hormone to capable binding sites. We now report specific, high affinity, low capacity binding of radioiodinated prolactin to cultured rat anterior pituitary cells, from two sources—those derived from oestradiol-induced anterior pituitary tumours, and normal anterior pituitary glands. Both cell lines were shown by radioimmunoassay to secrete prolactin. In addition to the possible significance of these findings with respect to pituitary control mechanisms they indicate the recommendation of the development of radioreceptor assays based on cultured cell lines as a source for specific binding sites. The technology seems to be available to develop a standardised assay of great convenience and versatility, which models better the conditions for hormone action *in vivo*; the regulatory hormone 'sees' only the outside of an intact cell in a favourable environment for specific interaction.

To study the prolactin-specific binding sites we used established clones of rat anterior pituitary cells (RAP) derived from oestrogen-induced pituitary tumours in Wistar-Furth rats, and primary cultures of normal anterior pituitary gland cells (RPG) from Sprague-Dawley rats. RAP lines were established and characterised as before¹¹. RPG cultures can be initiated and maintained in the same kinds of media¹¹ for as long as 40 d. From the tumour cell lines, clones C₁3RAP, C₂9RAP and C₈11RAP were singled out to determine the binding characteristics of ovine 125 I-prolactin (125 I-OPRL) in direct competition with excess unlabelled prolactin (OPRL) or, as a control, the non-lactogenic polypeptide, ovine luteinising hormone (OLH). The procedures for radioiodination and binding reactions have been described in detail¹² (see also legend to Fig. 1). Specific binding of prolactin was calculated as the difference in 125 I-OPRL bound in the presence of excess OLH (total bound) minus the 125 I-OPRL bound in the presence of excess OPRL (nonspecific).

Table 1 Competitive binding of ^{125}I -OPRL compared with OPRL and OLH by cultured rat anterior pituitary cells and centrifuged fractions

Source and treatment	A ^{125}I -OPRL unbound (d.p.m. per 400 μl medium)	B Total bound ^{125}I -OPRL (with 5 $\mu\text{g ml}^{-1}$ o-LH)	C Displaced ^{125}I -OPRL (with 5 $\mu\text{g ml}^{-1}$ o-PRL)	D Specifically bound Unbound
C₈11RAP cells				
Not frozen	140 3,856 36,427	209 $\pm$ 68 (3) 4,144 $\pm$ 1321 (5) 13,573 $\pm$ 5318 (4)	119 $\pm$ 16 (3) 2,798 $\pm$ 517 (5) 6,902 $\pm$ 575 (4)	0.84 0.73 0.19
Frozen	300 4,652 17,099 74,156	350 $\pm$ 46 (29) 5,348 $\pm$ 397 (48) 12,201 $\pm$ 2139 (3) 15,884 $\pm$ 1376 (16)	232 $\pm$ 29 (38) 3,098 $\pm$ 344 (41) 6,121 $\pm$ 678 (3) 8,354 $\pm$ 1311 (13)	0.77 0.67 0.22 0.11
C₃3RAP cells	9,512	488 $\pm$ 108 (15)	0 (18)	0
C₂9RAP cells	48,543	1,457 $\pm$ 178 (15)	0 (25)	0
RPG				
Not frozen	2,179 3,244 11,196 42,389 55,057	2,321 $\pm$ 646 (19) 3,756 $\pm$ 934 (14) 18,877 $\pm$ 843 (3) 47,611 $\pm$ 6902 (16) 73,043 $\pm$ 1071 (6)	1,878 $\pm$ 842 (19) 3,028 $\pm$ 745 (14) 6,013 $\pm$ 934 (3) 8,222 $\pm$ 980 (16) 9,023 $\pm$ 1805 (6)	0.65 0.58 0.54 0.19 0.11
Frozen	5,582 14,162 51,409 56,462 80,709	4,418 $\pm$ 953 (10) 12,138 $\pm$ 2150 (3) 33,524 $\pm$ 5422 (4) 33,574 $\pm$ 4409 (3) 49,293 $\pm$ 7964 (2)	3,651 $\pm$ 489 (10) 6,146 $\pm$ 940 (3) 7,805 $\pm$ 1842 (4) 8,305 $\pm$ 4899 (3) 9,041 $\pm$ 5949 (2)	0.65 0.43 0.15 0.15 0.11
1.0 M sucrose fractions				
Wistar-Furth adenohypophysis	633 4,259	367 $\pm$ 114 (10) 1,741 $\pm$ 202 (10)	179 $\pm$ 76 (10) 686 $\pm$ 141 (10)	0.28 0.16
KES mammary tumour cells	4,855 9,850	1,145 $\pm$ 214 (5) 2,140 $\pm$ 193 (4)	315 $\pm$ 112 (5) 452 $\pm$ 66 (4)	0.06 0.05
Lactating mouse mammary cells	2,882 8,474	2,118 $\pm$ 198 (7) 6,526 (4)	1,920 $\pm$ 134 (7) 4,685 $\pm$ 436 (4)	0.67 0.55

Iodinated prolactin in varying amounts was incubated at 4 °C for 30 min with 2×10^6 cells (100 μg protein) from rat anterior pituitary cell cultures or 100 μg of protein from 1.0 M sucrose fractions of isopycnic centrifugates of tissue homogenates (as a control for binding ability of the ^{125}I -OPRL preparations) in polyethylene microtubes with 200 μl of an incubation medium consisting of 1% BSA (bovine serum albumin, Sigma fraction V) 2mMNaCl and 2mM Tris-HCl buffer, pH 7.0. In alternate microtubes were 5 μg ml of OLH (column B) or OPRL (column C) in a final volume of 400 μl . The reactions were stopped by precipitating at 10,000g for 5 min (Beckman Microfuge 250), the supernatants were discarded and the surfaces of the pellets were washed by quickly adding and aspirating distilled water, thus diluting the extraneous film of ^{125}I -OPRL and bovine serum albumin. After the tips of the microtubes were cut off into 15 ml gamma counting tubes the precipitates were redistributed in 5 ml Lowry ^{14}C solution and counted for 20 min or until 10,000 c.p.m. (2% counting accuracy) had accumulated in the analyser circuits of a Beckman Model 1185 gamma counter (50% counting efficiency). Subsequently, the protein concentrations were determined and data expressed as bound d.p.m. per 100 μg protein- ^{125}I -OPRL complex counted $\pm$ s.d.(n). n, Number of experiments; represents average of five microtubes per experiment. The inhibition of specific binding by OPRL (displaced ^{125}I -OPRL; column C) was calculated by subtracting nonspecific from total counts (column B) and was significantly different in all cases except for experiments using C₃3RAP and C₂9RAP tumour cells.

^{125}I -OPRL bound in a rapid, specific and saturable manner with RPG, C₈11RAP cloned pituitary tumour cells (Fig. 1) and partially purified homogenates of normal rat anterior pituitaries, of a rat mammary tumour and of normal mouse mammary glands (Table 1). With cultured cells of two clones from different anterior pituitary tumour cell lines (C₃3RAP and C₂9RAP), there was no specific binding. We have shown as before³, that specific binding of ^{125}I -OPRL is proportionately greater than nonspecific binding, especially at low temperatures and at minimal concentrations of ^{125}I -OPRL; therefore we did most binding experiments at 4 °C and with low concentrations of added ^{125}I -OPRL while keeping the concentration of cells at about 2×10^6 (approximately 100 μg protein). We do not intend here to compare the affinities for prolactin of the various cells relative to those with sites of the highest affinity, nor to analyse whether one can detect a changing affinity for prolactin by the highest affinity complexes (co-operativity). More extensive experimentation is required to make these distinctions, but the preliminary evidence indicates that cultured RAP can be used to advantage in this regard.

The conditions of our experiments required that most of the clones of RAP would be collected and immediately washed twice with Hank's buffered salt solution (HBSS) and frozen at -180 °C. As freezing cultured cells may affect their ability

to bind specifically with prolactin, we compared the specific binding of frozen and unfrozen cells of C₈11RAP and RPG lines. Freezing and thawing once may reduce the number of specific binding sites slightly without changing the affinity of the remaining sites (compare curve in Fig. 1). We have observed, however, that repeated freezing and thawing (two to five times) of any of these rat pituitary cells or partially purified mouse mammary fractions progressively destroys their ability specifically to bind ^{125}I -OPRL. The lability of these binding sites attests to a steric requirement (proteinaceous) that is found in the binding sites for all other hormones yet reported.

For radioreceptor assay cultured cells may prove to be the most feasible source of highly specific binders. The conveniences of storing and shipping frozen cells most likely will compensate for the loss of some of the high affinity binding. As few as half a million cells per reaction can be used to assay an unknown concentration of prolactin in about 6 h. There is further question regarding the apparent loss of binding capacity by cells of lines cultured from the same oestradiol-induced pituitary tumours. Among possible explanations of our results we consider that cells that show specific binding in normal pituitary glands may represent all the cells present there, because when stimulated by oestradiol, chromophobes and growth hormone-secreting cells may secrete prolactin¹³.

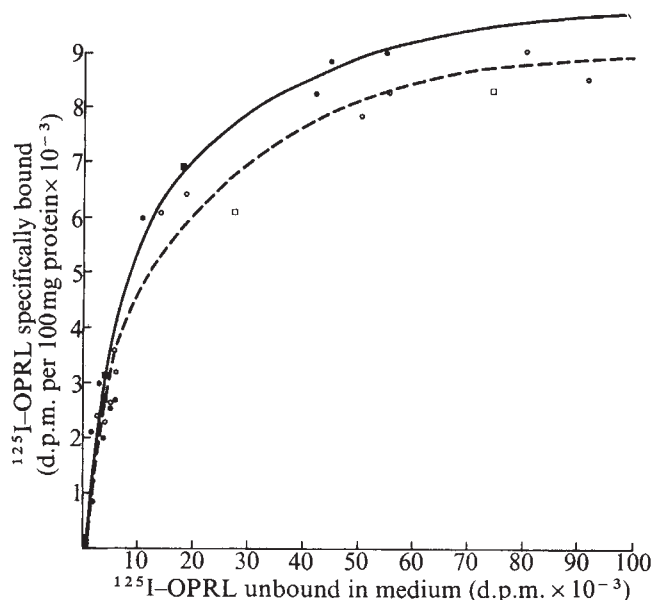


Fig. 1 NIH-S-12 OPRL in 100 μ l 40 mM barbital buffer, pH 7.0, was reacted with Na^{125}I (IMS-30, Amersham Searle) in a ratio of 5 μ g to 0.4 mCi in the presence of 10 μ g bovine lactoperoxidase (Sigma) and 0.25 μ g H_2O_2 (Mallinkrodt) for 2 min. The reaction was stopped by washing this mixture on to a Sephadex G-75 column (Pharmacia) previously equilibrated with 20 mg bovine serum albumin (BSA) and washed with a large excess of barbital buffer solution. The iodinated prolactin, eluted in the void volume of 40 mM barbital buffer, pH 7.0, was transferred to a column of DEAE-cellulose (diethylaminoethyl-cellulose, Bio-Rad) and washed with 10 volumes of 5 mM potassium phosphate (PK) buffer, pH 7.0. Elution was initiated using a 5–500 mM PK buffer gradient. The fraction shown previously¹³ to coelute with OPRL in identical column and buffer treatments was recovered and stored at 4 °C for use in the binding reactions. The second chromatographic step reduces the specific activity of this fraction to approximately 50 $\mu\text{Ci } \mu\text{g}^{-1}$ of prolactin but the favourable yield of biologically active ^{125}I -OPRL achieved by eliminating unbindable, radioiodinated products accounts for the high specific:free binding ratios. Increasing amounts of ^{125}I -OPRL (0.5 pmol d.p.m.⁻¹) was reacted with 2×10^6 cells (100 μ g protein) in a final volume of 400 μ l incubation medium (1% BSA, 2 mM NaCl and 2 mM Tris-HCl buffer, pH 7.0) at 4 °C for 30 min. In tubes used to determine total binding the competitor hormone was 5 $\mu\text{g ml}^{-1}$ OLG; for the determination of nonspecific binding the competitor hormone was 5 $\mu\text{g ml}^{-1}$ OPRL. Specific binding is calculated as the difference between total binding and non-specific binding. Solid line, specific binding for fresh (not frozen) cells of both C₈11 RAP and RPG (●) cultures; broken line, once-frozen and thawed cells of the C₈11 RAP (□) and RPG (○) cultures. The specific binding curves show that saturable binding occurs, but that there is less binding capacity for the once-frozen and thawed cells.

Probably, all prolactin-secreting and non-secreting cells (but which are potentially able to secrete prolactin) carry such binding sites; these binding sites may become the first step in a series of reactions tending to the control of prolactin secretion. On the other hand, the results with the oestradiol-induced pituitary tumour cells adapted to grow in culture and subsequently cloned suggest that only some cells within the total population can carry such binding sites. These cells eventually become prolactin-secreting when the proper stimuli for an increase in the synthesis and secretion of prolactin (oestradiol or oestradiol-dependent regulators) are applied¹³. We cannot rule out the possibility that all pituitary tumour cells have prolactin-binding sites but that some of them may have lost them while becoming established in culture. We are investigating these possibilities using cultured cells which model better the conditions for polypeptide hormone action *in vivo*.

W.L.F. was supported by a grant from the US National Science Foundation. C.S. was supported by grants and a

research career development award from NIH. We thank G. Murphy and M. Fong for assistance.

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Possible pathway for formation of fibrin degradation products during anecrod therapy

SOLUBLE complexes composed of protein antigenically related to fibrinogen have been found in the plasma of patients suffering from thrombosis or a hypercoagulable state¹. These complexes are important as they could provide a sensitive method for the laboratory diagnosis of such conditions at a very early stage in their development¹. Little is known concerning the structure, formation and breakdown of soluble complexes *in vivo*.

Soluble complexes can be produced by an intravenous infusion of thrombin in rabbits². Although such experiments are not possible in man, the use of thrombin-like snake venoms as defibrinating agents could provide a valuable alternative. Soluble complexes have been identified in the plasma of patients given defibrinating doses of the partially purified venom of *Bothrops moojeni* (Defibrase)³.

We have studied seven patients receiving treatment with anecrod, a defibrinating agent derived from the venom of *Agkistrodon rhodostoma*. *In vitro* anecrod resembles thrombin in removing fibrinopeptide A from fibrinogen but differs in failing to remove fibrinopeptide B (ref. 4). *In vivo* anecrod infusion is thought to produce rapid defibrination by the formation of insoluble fibrin microclots throughout the circulation, which are removed by fibrinolysis and reticulo-endothelial cell activity⁵.

Anecrod (Arvin, Berk, Shalford, Surrey, UK) was administered intravenously in a dose of 2 U per kg body weight per 12 h. Blood samples were taken before starting treatment and after 6 and 24 h therapy. Plasma was obtained using trisodium citrate (final concentration 0.013 M) as anticoagulant. Epsilon aminocaproic acid (EACA), aprotinin and anecrod antiserum were added to prevent any *in vitro* proteolysis. Samples were tested immediately without storage, as freezing and thawing may cause the formation of soluble complexes⁶.

Soluble complexes were separated from uncomplexed fibrinogen-related protein by 6% agarose gel filtration

(Biogel A-5m, BioRad Laboratories, 1510750) of a β alanine precipitate of plasma^{1,7}. EACA and aprotinin were added to the elution buffer. Protein-containing eluant fractions were analysed for fibrinogen-fibrin related antigen (FR-antigen) by a radial immunodiffusion technique⁸ using fibrinogen antiserum (Behringwerke-Hoechst, ORCH 05). There may be changes in the antigenic structure of the fibrinogen-related molecules when incorporated into soluble complexes and their diffusion properties may also be altered. This method is, therefore, only semiquantitative but changes in the distribution of FR-antigen were clearly shown and the results correlated well with those obtained by continuous absorbance monitoring at 280 nm.

FR-antigen from the plasma of normal control subjects eluted from the column as a symmetrical curve with a peak at 90 ml. The results from a representative patient are shown in Fig. 1. Before starting treatment there is an essentially normal curve but after 6 h of ancrod infusion a range of soluble complexes were present extending from the void volume (60 ml) to the position of standard fibrinogen. Degradation products of lower molecular size were also present.

Polyacrylamide gel electrophoresis in sodium dodecyl sulphate and mercaptoethanol⁹ was performed to study the chain structure of the component units of the soluble complexes. Samples were taken from the complexes present at 60 and 80 ml and compared with the uncomplexed material at 90 ml. Where electrophoresis could not be performed immediately, samples were stored at -20°C and tested within 24–48 h. The results for two patients are shown in Fig. 2.

Fibrinogen is composed of three pairs of polypeptide chains ($\text{A}\alpha$, $\text{B}\beta$, γ). In all seven patients there was marked loss of intact $\text{A}\alpha$ chain in the soluble complex material. In six out of seven patients there was only minimal loss of intact $\text{A}\alpha$ chain in the main peak fraction (see W.McG.). The remaining patient (J.McK.) showed virtual absence of intact $\text{A}\alpha$ chain.

Proteolytic enzymes initially attack the $\text{A}\alpha$ chain of fibrinogen. The greater degree of $\text{A}\alpha$ chain loss in the soluble complexes in six out of seven patients suggests that

Fig. 1 Elution pattern of FR-antigen following agarose gel filtration of a β alanine precipitate of plasma obtained from one patient (W.McG.) before starting treatment (----) and after 6 (—) and 24 h (●) therapy.

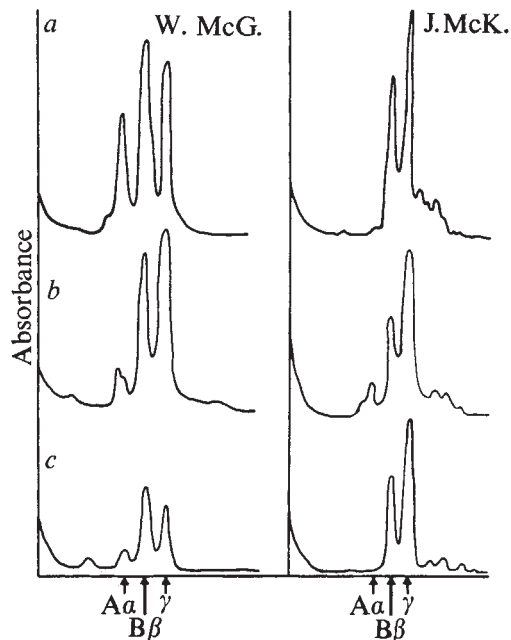
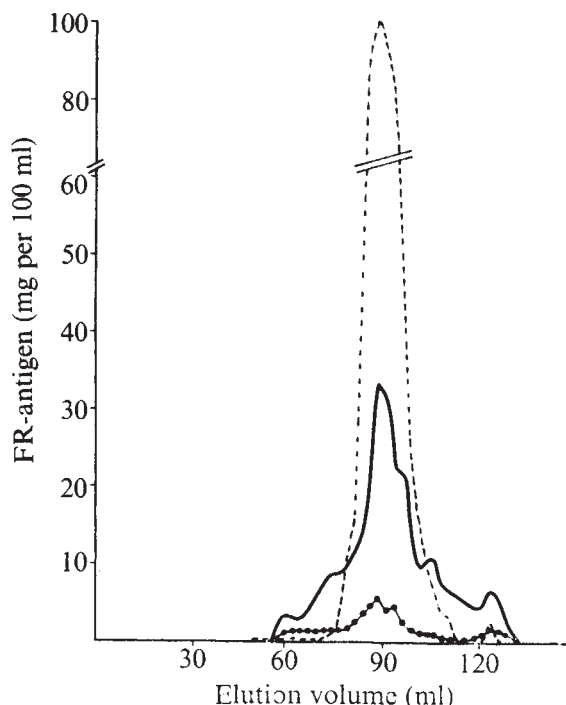


Fig. 2 Densitometric scans of polyacrylamide gel electrophoreses of selected fractions (a, main peak fraction at 90 ml; b, intermediate zone fraction at 80 ml; c, void volume fraction at 60 ml) from agarose gel filtration of the 6 h samples from two patients (W.McG. and J.McK.). The migration position of the $\text{A}\alpha$, $\text{B}\beta$ and γ chains of standard fibrinogen are indicated.

preferential proteolytic digestion of the complexes may have occurred. As there is marked plasminogen depletion during ancrod therapy⁷, plasmin may be responsible for this proteolysis. Ancrod can produce $\text{A}\alpha$ digestion *in vitro*¹⁰, but the concentration of ancrod required is much greater than that present therapeutically.

Soluble complexes deficient in intact $\text{A}\alpha$ chain could be produced by the repolymerisation of early degradation products² or directly by the lysis of insoluble fibrin⁷. It has also been suggested that soluble fibrin monomer is lysed by plasmin in preference to fibrinogen *in vitro*¹¹. If this occurred *in vivo* rapidly enough to prevent the formation of insoluble fibrin, soluble complexes similar to those demonstrated would be formed. Further digestion by plasmin would produce uncomplexed fibrin degradation products (FDP).

FDP in ancrod-treated patients may, therefore, be derived from soluble rather than insoluble fibrin. A similar suggestion has been made based on studies of the cross-linkage of the FDP (ref. 12). Microclots would not form within the circulation, explaining the benign defibrination state produced by ancrod infusion. The preferential proteolysis of soluble fibrin could represent an important pathway for the production of FDP *in vivo*.

We thank Mr D. W. Short for permission to study three patients with peripheral arterial disease, and Berk Pharmaceuticals Ltd for the gift of Arvin. C.R.M.P. is a Wellcome Research Fellow.

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Received April 22; accepted May 5, 1975.

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Defective microbicidal activity in glutathione peroxidase-deficient neutrophils of selenium-deficient rats

SEVERAL enzymes are involved in the microbicidal activity of phagocytes^{1–3}. Glutathione peroxidase (GSHPx) deficiency has been associated with decreased microbicidal ability in leukocytes from two female patients with chronic granulomatous disease, a rare hereditary disorder⁴. With the discovery that the essential trace element selenium (Se) is an integral part of GSHPx, it is now possible to induce experimental GSHPx deficiency by feeding animals a Se-deficient diet⁵. We report here an impairment in the killing of yeast cells ingested by GSHPx-deficient leukocytes from rats depleted of Se.

Male weanling rats were depleted of Se by feeding a basal diet⁶ adequate in all known nutrients except Se (less than 13 parts per 10⁹). Controls were fed the basal diet supplemented with 0.50 p.p.m. Se as sodium selenite. Blood samples were obtained by cardiac puncture. Peritoneal exudates were elicited by intraperitoneal injection, under light ether anaesthesia, of 1 ml sterile light mineral oil (Sargent-Welch, SC13637) and collected⁶ and resuspended 10–12 h later. Exudates consisted of more than 85% polymorphonuclear neutrophils (PMN) as shown by differential staining with Wright's stain. GSHPx activities⁷ of the 2,000g supernatants of freeze-thawed exudates from rats depleted for 5 weeks were 10–15% of control values. Activities⁸ of an enzyme which does not contain Se, superoxide dismutase, in the same exudates were not depleted in this way. Activities of GSHPx in whole blood were less than 1% of control values after 13 weeks; fluorometric analysis⁹ showed that Se in whole blood was 3% of control. After 17 weeks, leukocyte and differential counts of Se-deficient and control rats were similar and within the normal range.

Microbicidal assays¹⁰ facilitated direct microscopic observations (in PMN monolayers from whole blood) of the numbers of *Candida albicans* ingested or killed by each PMN. Viability was determined with methylene blue, which stains non-viable PMN and yeast but not living cells.

Fungicidal activity, whether expressed as a percentage or as the number of yeast cells killed per ingesting PMN, was considerably less in PMN from blood of Se-deficient rats than in those from controls (Table 1). There were no apparent differences in ingestive ability of PMN from Se-deficient or control animals. Table 2 shows the results of similar experiments carried out with peritoneal exudate cells from a few animals. Although none of the differences between Se-deficient and control animals was statically significant, the results were not inconsistent with those of Table 1. In experiments I, II and III (Table 2) the large differences in killing by exudate cells from rats of the same dietary group could be attributable to differences in collection and centrifugation conditions. Phagocytic activity of peritoneal phagocytes is dependent on such factors⁶.

Appropriate control experiments were included to determine whether the numbers of intracellular blue-stained *C. albicans* corresponded to the actual numbers of dead *C. albicans* with PMN. After peripheral PMN from either control or Se-deficient animals had ingested live (more than 99.5% viable) or heat-killed *C. albicans* for 10 min, and before killing could occur the PMN monolayers were

exposed to methylene blue. When live *C. albicans* were used, apparent killing was minimal: the percentage of stained intracellular yeast was approximately equal to that of stained extracellular yeast, which was less than 0.5%. When heat-killed *C. albicans* was used, all yeast cells stained blue whether inside or outside the PMN. Results were the same whether PMN were from Se-deficient or control animals.

Other control experiments confirmed the viability of *C. albicans* incubated in the absence of PMN, and the viability (more than 95%) of intact PMN from Se-deficient and control rats incubated in the absence of *C. albicans*. Intact PMN from rats in either dietary group retained their high viability, after ingestion and killing *C. albicans*, for 75 min. At the end of the incubation, no difference in PMN monolayer densities between dietary groups was apparent.

This technique for measuring fungicidal activity has been applied to clinical screening and has demonstrated the importance of myeloperoxidase to PMN^{10,11}. Our results represent a second example of a cellular defect in fungicidal activity (but not ingestive activity) observed using this technique to study both processes simultaneously in viable, intact phagocytes. To our knowledge, this is the first clear demonstration of impaired phagocytic microbicidal capacity linked to the deficiency of a specific nutrient.

During the formation of microbicidal metabolites by phagocytes, hydrogen peroxide is generated, probably within the phagolysosome¹². Peroxidation of ingested and endogenous lipid occurs in PMN and alveolar macrophages during phagocytosis¹³ and linoleic acid hydroperoxide inhibits the cytosolic sulphhydryl enzymes, 6-phosphogluconate dehydrogenase, glucose-6-phosphate dehydrogenase (G6PD) and glyceraldehyde-3-phosphate dehydrogenase in sonicated alveolar macrophages¹⁴. Such inhibition could interfere with normal phagocytosis, for deficiencies of leukocyte G6PD have been correlated with decreased leukocytic bactericidal activity and mild chronic granulomatous disease¹. Lipid hydroperoxides may also harm granule membranes, causing release of various granular hydrolases to the cytoplasm and the extracellular milieu. Such changes in hydrolase distribution have been observed in alveolar macrophages and whole lung tissue damaged by ozone¹⁵.

Table 1 Ingestion and killing of *C. albicans* by rat peripheral PMN*

Diet	-Se		+Se	
	Experiment I†	Experiment II	Experiment I	Experiment II
Ingestion: (yeast cells per PMN)	2.0 ± 0.2	2.0 ± 0.2	2.0 ± 0.1	2.4 ± 0.2
Killing:				
(a) Stained yeast cells per ingesting PMN	0.14 ± 0.04‡	0.15 ± 0.02§	0.45 ± 0.07	0.50 ± 0.04
(b) Stained yeast cells as percentage ingested yeast cells	6.6 ± 2.1†	7.2 ± 0.7§	22 ± 4	21 ± 1

*Techniques of Schmid and Brune¹⁰, with the following modification: Preincubation time 30 min instead of 60 min, ingestion time 15 min instead of 10 min, killing time 60 min instead of 50 min, total time 105 min instead of 120 min. Blood samples contained heparin. Serum was pooled from four Se-adequate rats not used for PMN collection. PMN monolayer densities were not controlled precisely but yeast density was sufficiently high ($2.5 \times 10^6 \text{ cm}^{-2}$) compared with PMN density (approximately $1 \times 10^6 \text{ cm}^{-2}$) for ingestion saturation after 10 min.

†Rats for experiments I and II were from same supplier (Holtzman) but were different lots placed on experiment at different times. They were depleted of Se for either 12 weeks (I) or 17 weeks (II). In each group, duplicate samples of cells were assayed from each of three animals (I) or five animals (II). Values shown are mean ± s.e.m.

‡Significantly different from corresponding control value ($P < 0.05$).

§Significantly different from corresponding control value ($P < 0.001$).

Table 2 Ingestion and killing of *C. albicans* by rat peritoneal exudate cells*

Diet	-Se	+Se
Experiment I†		
Ingestion: (yeast cells per PMN)	3.9 ± 1.0	4.6 ± 0.2
Killing:		
(a) Stained yeast cells per ingesting PMN	0.54 ± 0.03	0.93 ± 0.19
(b) Stained yeast cells as percentage of ingested yeast cells	14 ± 3	20 ± 5
Experiment II		
Ingestion:	2.8 ± 0.7	3.6 ± 0.1
Killing:		
(a) Stained yeast cells per ingesting PMN	0.22 ± 0.03	0.36 ± 0.01
(b) Stained yeast cells as percentage of ingested yeast cells	8.00 ± 0.9	10.1 ± 0.1
Experiment III		
Ingestion:	2.9 ± 0.1	3.5 ± 0.3
Killing:		
(a) Stained yeast cells per ingesting PMN	0.025 ± 0.001	0.098 ± 0.054
(b) Stained yeast cells as percentage of ingested yeast cells	0.87 ± 0.08	2.7 ± 1.3

*Preincubation time, 15 min; ingestion time 20 min; killing time, 30 min; staining time, 15 min. Yeast density, $2.0 \times 10^6 \text{ cm}^{-2}$; phagocyte density, $4.0 \times 10^4 \text{ cm}^{-2}$.

†Rats for experiments I, II, and III were from same lot and were fed for 17–18 weeks postweaning before cell collection. Collection and centrifugation of exudates were similar within experiments, but differed between experiments. In each dietary group, duplicate samples were assayed from one animal (I, II) or two animals (III). Values shown are mean $\pm$ s.e.m.

We suggest that GSHPx, by reducing lipid hydroperoxides (as well as hydrogen peroxide) to the corresponding alcohol and water, protects cytosolic components and membranes from reactive metabolites diffusing from the phagolysosome. Other enzymes that destroy peroxides, such as catalase, are not known to decompose lipid hydroperoxides¹⁶ and, unlike GSHPx, are not usually predominant in the cytosol. This suggested role for Se is based on the fact that GSHPx is the only mammalian enzyme known to contain Se, but it is entirely possible that some other selenoenzyme or other form of biologically active Se, not yet discovered, may be involved. Whatever the functional form of Se, viability of and ingestion by PMN seem to be unaffected, but fungicidal capacity seems to be altered. Perhaps this is an indication that production of killing metabolites is required for effects of Se to become evident.

We thank R. D. Hinsdill and coworkers for advice. This research was supported by grants from the National Institute of Health.

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Received February 24; accepted May 8, 1975.

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Tissue-mediated mutagenicity of vinylidene chloride and 2-chlorobutadiene in *Salmonella typhimurium*

CHLORINATED hydrocarbons, such as vinyl chloride or vinylidene chloride (1,1-dichloroethylene), are produced in large quantities and are present in the environment¹. Recently, vinyl chloride monomer has been shown to be carcinogenic in animals and man^{2–4} and mutagenic in microbial systems^{5–7}. Vinylidene chloride (VDC), a structurally related substance and co-polymer of vinyl chloride, is used in the manufacture of plastics⁸; it could also occur as a decomposition product of 1,1,1-trichloroethane¹. Another chemically related compound, 2-chlorobutadiene (chloroprene), has been used in the manufacture of synthetic rubber since 1930.

We have examined the mutagenicity of VDC and 2-chlorobutadiene in *Salmonella typhimurium* strains, using a tissue-mediated assay which has been found effective in detecting the mutagenicity of various carcinogens, such as nitrosamines⁹, vinyl chloride⁶ and many others^{10,11}.

Tests for mutagenicity were carried out according to the procedure developed by Ames *et al.*¹¹ and modified by Bartsch *et al.*⁵ using the histidine-auxotroph strains of *S. typhimurium* TA1530 and TA100, provided by Professor B. N. Ames. In this procedure, Petri dishes containing 9,000g tissue supernatant, an NADPH-generating system and the bacteria in a soft agar layer were exposed to various concentrations of VDC or 2-chlorobutadiene vapour in a desiccator at 37 °C in the dark. The compounds were then replaced by air, and, after further incubation for up to 48 h at 37 °C, the number of histidine-revertant colonies per plate was counted. The concentration of VDC or 2-chlorobutadiene in the incubation medium was determined by gas-liquid chromatography⁶. In the experimental conditions used, the concentrations of VDC in the aqueous phase after 2 h of exposure to 0.2, 2 or 20% VDC in air (v/v) were 3.3×10^{-4} M, 3.3×10^{-3} M or 3.3×10^{-2} M, respectively. The concentrations of 2-chlorobutadiene in the aqueous phase after 2 h of exposure to 0.5, 2 or 8% vapour in air (v/v) were 7×10^{-5} M, 3.7×10^{-4} M or 1.65×10^{-3} M, respectively. No further increases were observed after up to 7 h of exposure.

Fig. 1 shows the mutagenic effect on *S. typhimurium* TA1530 and TA100 of exposure to 0.2, 2 or 20% VDC in air in the presence of 9,000g liver supernatant of phenobarbitone-pretreated mice (0.1% in the drinking water for 7 d). When the cofactors (NADP⁺ and glucose-6-phosphate), required for activity of microsomal mixed-function oxidase, were omitted from the incubation mixture, no mutagenic effect was observed. The mutagenic response, which was greater in the TA100 strain, increased in both strains after exposure to up to 2% VDC in air. The lower mutagenic response observed with a concentration of 20% VDC may result from an inhibitory action of VDC and (or) its metabolite(s) on the microsomal enzymes responsible for the metabolic activation of VDC. 4-Methoxyphenol, which is used as a stabiliser of VDC, when applied at up to 500 µg per plate, was not mutagenic for either strain, whether in the presence or absence of microsomal liver fraction from phenobarbitone-pretreated mice.

Exposure of TA100 strain to 2% VDC in air in the presence of a hepatic microsomal fraction from untreated or phenobarbitone-pretreated mice, caused a linear increase in mutagenic response up to 4 h. Thus, exposure to 2 or 20% VDC in air for 4 h was used to assay rat and mouse liver, kidney and lung fractions (9,000g supernatant) for their ability to convert VDC into mutagenic metabolites (Table 1).

Mouse liver, kidney and lung fractions efficiently converted

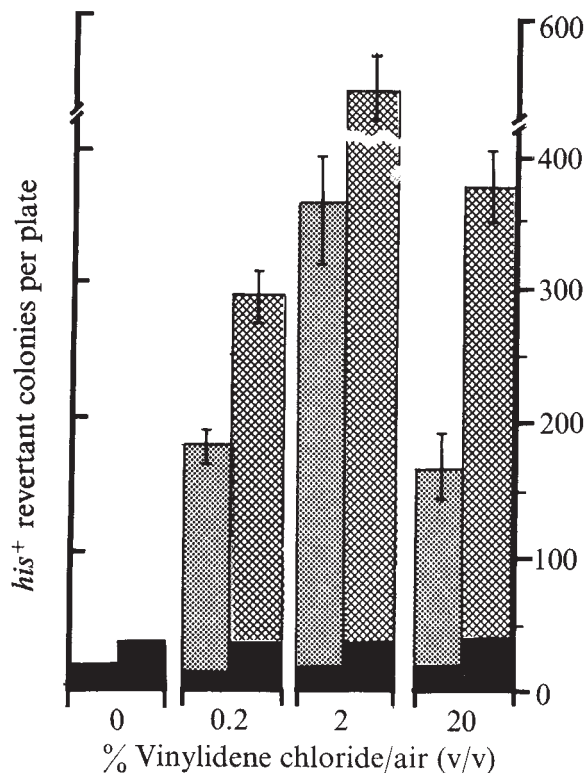


Fig. 1 VDC concentration-dependent induction of reverse mutations in *S. typhimurium* strains TA1530 and TA100. Petri dishes containing 9,000g liver supernatant from phenobarbitone-pretreated male OF-1 mice, NADP⁺ (2 μ mol per plate), glucose-6-phosphate (2.5 μ mol per plate) and the bacteria in a histidine-deficient medium, were exposed to 0, 0.2, 2 or 20% VDC in air (by volume) for 4 h in a desiccator at 37 °C in the dark. VDC was removed under vacuum and replaced by air, and the incubation was continued up to 48 h at 37 °C. Bacterial survival was estimated by seeding TA1530 (10⁻⁷ dilution) on a histidine-enriched medium. Control assays were carried out by omitting the cofactors (NADP⁺, glucose-6-phosphate). Mean values $\pm$ s.e. from 1-4 series of experiments, each on a pool of five mouse livers, are plotted. Bacteria were plated in triplicate. VDC, containing 0.3% 4-methoxyphenol as antioxidant, was obtained from Merck-Schuchardt (Darmstadt, Federal Republic of Germany). Black: control; stippled, TA 1530; cross-hatched, TA100.

VDC into mutagenic metabolites *in vitro*, activity being greatest in the liver. Phenobarbitone pretreatment of the mice produced an increase in the mutagenic response in assays with all three organ fractions. A much lower mutagenic response was observed in rats than in mice with liver and kidney fractions, and there was only minimal activity with a lung fraction. Rats have been reported to show hepatic and renal damage following inhalation of VDC (ref. 12). When the concentration of VDC in air was raised from 2 to 20%, an increased mutagenic effect was noted only with mouse kidney and lung fractions.

As has been shown previously for vinyl chloride, these results demonstrate that the mutagenic effect of VDC is mediated by microsomal enzymes from various organs, in the presence of an NADPH-generating system and oxygen; these enzymes probably form the alkylating intermediate(s), since *S. typhimurium* TA1530 strain is specifically reverted by monofunctional alkylating agents¹¹. Evidence for the participation of a microsomal mixed-function oxidase is further supported by the observation of changes in mutagenicity *in vitro* and (or) the toxicity of VDC following pretreatment of rats or mice with various drugs which are known to modify the activity of drug-metabolising enzymes. As shown in Table 1, phenobarbitone pretreatment of mice caused an increased mutagenic response. In another series of experiments, we examined the effects of pretreating female BD-VI rats with phenobarbitone (0.1% in the drinking water for 7d), pregnenolone-16 α -carbonitrile (PCN; 50 mg kg⁻¹; 5 times orally at 12 h intervals) or aminoacetonitrile (AAN; 500 mg kg⁻¹, one subcutaneous injection 24 h before the assay) on the mutagenic effect of exposure to 2% VDC in air on *S. typhimurium* TA1530 strain with liver fractions in assays as described in Fig. 1. Phenobarbitone caused a twofold increase in the mutagenic response, whereas PCN or AAN resulted in reductions of 40 or 60%, respectively, when compared with the corresponding values obtained from untreated rats. Carlson and Fuller¹³ reported an increased mortality by VDC in rats following phenobarbitone pretreatment.

The addition of sulphur-containing compounds to *in vitro* mutagenicity assays containing mouse-liver fractions reduced the mutagenic effect of exposure to 2% VDC in air on the *S. typhimurium* TA1530 strain. Addition of N-acetyl-cysteine and N-acetyl-methionine (12 μ mol of each per ml soft agar layer) caused an 80% reduction of mutagenic response compared with the appropriate control. These results indicate that

Table 1 Mouse and rat tissue mediated mutagenicity of VDC in *S. typhimurium* TA100*

Experiment no.	Species	Phenobarbitone pretreatment	Tissue (9,000g supernatant†)	Cofactors‡	2% VDC in air his ⁺ revertants per plate§	Relative activity	20% VDC in air his ⁺ revertants per plate§	Relative activity
1	OF-1 mouse♂	Yes	Liver	+	500 $\pm$ 23	150	330 $\pm$ 29	75
2		Yes		+	23 $\pm$ 10	5	7 $\pm$ 5	2
3		No		+	330 $\pm$ 49	100	435 $\pm$ 46	100
4		No		-	16 $\pm$ 4	5	1 $\pm$ 3	0
5		Yes		+	147 $\pm$ 15	45	173 $\pm$ 5	40
6		Yes		-	31 $\pm$ 7	9	17 $\pm$ 2	4
7		No	Kidney	+	67 $\pm$ 2	20	125 $\pm$ 5	29
8		No		-	20 $\pm$ 3	6	16 $\pm$ 1	4
9		Yes		+	34 $\pm$ 4	10	48 $\pm$ 5	11
10		Yes	Lung	-	5 $\pm$ 4	1	10 $\pm$ 1	2
11		No		+	21 $\pm$ 5	6	37 $\pm$ 3	8
12		No		-	6 $\pm$ 9	2	14 $\pm$ 8	3
13	BDVI rat ♀	No	Liver	+	95 $\pm$ 7	30	77 $\pm$ 5	18
14		No		-	0	0	2 $\pm$ 2	0
15		No		+	18 $\pm$ 4	5	16 $\pm$ 2	4
16		No	Kidney	-	18 $\pm$ 2	5	21 $\pm$ 4	5
17		No		+	9 $\pm$ 2	3	11 $\pm$ 2	3
18		No		-	9 $\pm$ 7	3	12 $\pm$ 6	3

*Assays carried out as described in Fig. 1.

†Equivalent to 38 mg wet tissue per plate.

‡NADP⁺ (2.0 μ mol per plate) and glucose-6-phosphate (2.5 μ mol per plate).

§Mean values $\pm$ s.e. from 1-4 experiments, each using pooled tissues from 4 mice or 3 rats. The number of spontaneous mutations per plate (49 $\pm$ 2) has been subtracted from each value.

||Relative mutagenic activity was expressed by taking the value obtained in experiment 3 as 100.

Table 2 Mutagenicity of 2-chlorobutadiene in *S. typhimurium* TA100*

Experiment no.	2-chlorobutadiene† in air (%)	Phenobarbitone pretreatment	<i>his</i> ⁺ revertants/plate 9,000g liver supernatant + cofactors‡	KCl§
1	0	Yes or no	35 ± 3	35 ± 2
2	0.5	Yes	175 ± 15	49 ± 3
3		No	101 ± 4	
4	2	Yes	232 ± 17	70 ± 4
5		No	154 ± 15	
6	8	Yes	331 ± 30	117 ± 6
7		No	306 ± 25	

*Bacteria were exposed to 0.5, 2 and 8% 2-chlorobutadiene in air (v/v) for 4 h at 37 °C in presence of a NADPH generating system and a 9,000g liver supernatant from either phenobarbitone-treated or untreated male OF-1 mice as described in Fig. 1.

†2-Chlorobutadiene (purity 98.94%) was provided by Distugil, Le Pont de Claix, France, contaminated with 0.98% 1-chlorobutadiene, 370 p.p.m. butadiene and 280 p.p.m. vinylacetylene. Tertiary butylcatechole which was present in 2-chlorobutadiene as an antioxidant (200 p.p.m.) was not mutagenic up to 500 µg per plate for the TA100 strain either in the presence or absence of a microsomal liver fraction of phenobarbitone pretreated mice.

‡When the cofactors (NADP⁺ and glucose-6-phosphate) were omitted, the number of *his*⁺ revertants per plate was identical with assays containing KCl only. Mean values ± s.e. from 2 series of experiments, each on pooled livers from 4 mice.

§9,000g supernatant + cofactors was replaced by a 0.9% KCl solution.

the mutagenic VDC metabolite(s) is trapped by nucleophilic sulphur groups, thus competing for binding to bacterial DNA. This observation parallels the findings of Jaeger *et al.*¹⁴ who observed that the toxicity of VDC in rats is correlated with hepatic glutathione concentration, and that cysteine has a protective effect.

Chloroethylene oxide, the suggested primary metabolite of vinyl chloride, has been shown to be an alkylating and mutagenic agent⁶. Since epoxides are now recognised as obligatory intermediates in the metabolism of olefinic compounds by hepatic microsomal mixed function oxidase¹⁵, it can be postulated that 1,1-dichloroethylene oxide may be a primary reactive metabolite of VDC. It is also possible that partial dechlorination¹⁶ of VDC by microsomal enzymes results in vinyl chloride and its metabolic products.

Exposure of *S. typhimurium* TA100 strain to 0.5% up to 8% of 2-chlorobutadiene in air in the absence of any metabolic activation system caused a linear increasing mutagenic response, as a function of the 2-chlorobutadiene concentration, which, at a concentration of 8%, reached three times the spontaneous mutation rate (Table 2). Exposure to higher concentration (20%) caused a strong toxicity in the bacteria. This mutagenic and (or) toxic effect could be caused by a direct action of 2-chlorobutadiene or, more likely, by one of its enzymic (bacteria) or non-enzymic breakdown products. A three or twofold increased mutagenic response, respectively, was observed when a fortified 9,000g liver supernatant from either phenobarbitone or untreated mice was added to such assays (Table 2). These results support an enzymic formation of a mutagenic metabolite(s) from 2-chlorobutadiene, probably an epoxide, as it was strongly implicated for vinyl chloride⁶.

Most chemical carcinogens have now been found to be mutagens, when assayed in one of the mutagenicity tests that combine microbial or mammalian cell systems as genetic targets with an *in vitro* or *in vivo* metabolic activation system¹⁷. The increasing evidence of a possible correlation between mutagenicity and carcinogenicity does not mean that one biological effect may be equated with another. Thus, mutagenicity in microorganisms cannot be automatically assumed to imply a possible carcinogenic effect in man. Mutagenicity tests are at present an excellent method for screening environmental chemicals to be submitted to a more careful set of bioassays. While there are no criteria at present for extrapolation from experimental data to man, the available evidence indicates that precautions are justifiable on the basis of experimental evidence of carcinogenicity. There is, in fact good indication that experimental evidence of carcinogenicity can in certain cases predict a similar effect in

man; as was the case of diethylstilboestrol, *bis*-chloromethylether and vinyl chloride¹⁸.

Recently, VDC has been found to be carcinogenic in the rat, (Viola, Bigotti and Caputo, personal communication) and 2-chlorobutadiene has been suggested as being responsible for an increased incidence of skin and lung cancer among exposed workers¹⁹. A high incidence of chromosome aberration in lymphocytes from the peripheral blood of workers exposed to 2-chlorobutadiene has been reported²⁰. These findings, combined with the present results, indicate that an epidemiological survey of workers exposed to these substances may be necessary, as well as measures to prevent, or greatly reduce, exposure of humans.

We thank Mrs C. Gabet and Mr A. Barbin for technical assistance. This research was partially supported by a contract with the US National Cancer Institute.

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Received April 11; accepted May 7, 1975.

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Melanocyte-stimulating hormone promotes activation of pre-existing tyrosinase molecules in Cloudman S91 melanoma cells

TYROSINASE activity is greatly enhanced in cultured Cloudman S91 melanoma cells following addition of melanocyte-stimulating hormone (MSH) to the culture medium¹. The increased activity occurs in the G2 phase of the cell cycle² because membrane receptors for MSH are available only in this phase³. The response to MSH is mediated through cyclic AMP (refs 1-4). It is well documented that many peptide hormones function by activating membrane-bound adenyl cyclase molecules, causing net increases in intracellular cyclic AMP concentrations. These increases in turn have profound effects on cell division, morphology, and the expression of differentiated functions in a wide variety of cells and tissues. Little is known, however, about the levels at which genetic expression is regulated or the molecular intermediates involved in the regulation. For example, it is generally assumed that cyclic AMP-dependent protein kinases are involved in many of the responses because most tissues contain such enzymes⁵. The regulation of glycogenolysis is the best known example supporting this assumption⁶⁻⁷. But it cannot be taken as fact that

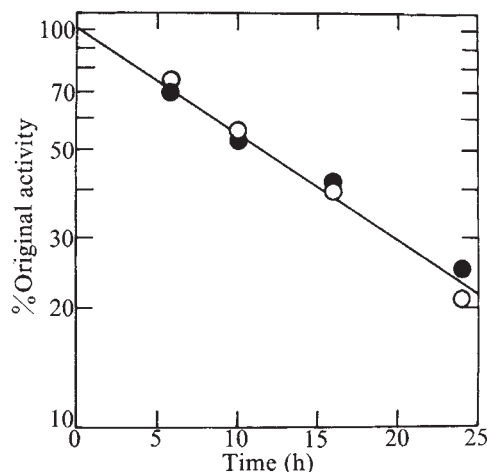


Fig. 1 Decay of tyrosinase activity. Cells (10^6) were grown in normal culture medium or medium containing MSH (2×10^{-7} M) for 48 h. At that time cycloheximide (10^{-6} M) was added to stop new protein synthesis and tyrosinase activity was measured as a function of ^3HOH production for the next 24 h. The results represent averages from triplicate culture flasks, and are the percentage of the tyrosinase activity seen in the corresponding control cells which received no cycloheximide.

Table 1 Effects of actinomycin D and cycloheximide on tyrosinase activity in synchronised cells

Treatment	^3HOH production (c.p.m. per 10^6 cells)	
	Period after release from colchicine 0-24 h (predominantly G1-S)	24-48 h (predominantly G2)
Culture medium only	1,289	1,842
+ MSH	1,174	29,706
+ MSH + actinomycin D	0	26,568
+ MSH + cycloheximide	1,850	27,900

Cells were arrested in metaphase with colchicine, collected by manual shaking², and 10^6 inoculated into 30 ml Falcon tissue culture flasks containing 5 ml F10 culture medium, $1 \mu\text{Ci ml}^{-1}$ $5\text{-}^3\text{H-L-tyrosine}$ (New England Nuclear, final specific activity in medium 90 mCi mmol^{-1}) and MSH (2×10^{-7} M), actinomycin D ($0.05 \mu\text{g ml}^{-1}$), or cycloheximide (10^{-6} M). Additions were made at $t = 0$ or $t = 24$ h after release from colchicine, and tyrosinase activity was measured as a function of ^3HOH production in 1 ml culture medium⁸ after the first or second 24 h of the cell cycle. Results represent averages from triplicate culture flasks, and the experiments were repeated several times with similar results each time.

in eukaryotes all cyclic AMP-mediated processes are post-translational in nature.

In this report we present evidence that at least one control exerted by cyclic AMP in melanoma cells is indeed at the post-

translational level, in that MSH seems to act by promoting the conversion of pre-existing tyrosinase molecules from an inactive to an active state. The reaction probably involves the inactivation of an inhibitor of the enzyme. The findings which lead us to these conclusions are as follows: tyrosinase activity increases following the addition of MSH in the absence of RNA and protein synthesis, suggesting that the response to the hormone does not require the synthesis of new molecules of the enzyme; MSH does not prolong the half life of tyrosinase in the cells; crude extracts of cells not treated with MSH contain factors which inhibit tyrosinase activity in extracts of cells which have been treated with the hormone, indicating that MSH promotes the inactivation of these factors; and by purifying tyrosinase from the cells we found that there were the same number of molecules of the enzyme whether or not the cells were pretreated with MSH.

Cells were synchronised by exposure to colchicine, and those in metaphase were collected by manual shaking². One complete cell cycle was about 48 h in length and the cells were exposed to MSH, actinomycin D, or cycloheximide for the first 24 h (predominantly G1-S) or second 24 h (predominantly G2) of the cycle. Tyrosinase activity was measured during each time period. The concentrations of actinomycin D and cycloheximide were sufficient to inhibit RNA and protein synthesis respectively by more than 95%. Tyrosinase activity increased in response to MSH during the G2 phase of the cycle and the increase was

Table 2 Tyrosinase activity in mixtures of various extracts

Source of extract	Additive (1:1)	Tyrosinase activity (c.p.m. ^3HOH production)
(a) 20,000g supernatant from control cells	Buffer	3,500
(b) 20,000g supernatant from MSH-treated cells	Buffer	32,100
(c) Supernatant from MSH-treated cells	Supernatant from control cells	9,300
(d) Tyrosinase purified 1,000-fold from Sephadex-tyrosyl-tyrosine column	Buffer	150,000
(e) Tyrosinase purified 1,000-fold	20,000g supernatant from control cells	13,800

Supernatants (20,000g) were prepared from 5×10^6 cells grown for 48 h in normal culture medium or medium containing MSH (2×10^{-7} M). Buffer was 5 mM NaPO_4 , pH 6.8. Purification of tyrosinase on Sephadex-tyrosyl-tyrosine is described in Table 3. ^3HOH was measured after incubation of the various extracts with $^3\text{H-tyrosine}$ for 1 h at 37°C .

Table 3 Purification and quantification of soluble Tyrosinase

Purification steps		Units of activity	Specific activity U per mg protein	c.p.m. $\times 10^6$ in ^3H -labelled proteins	c.p.m. $\times 10^3$ in ^{14}C -labelled tyrosinase standard
20,000g supernatant	C	200	7.0	44.0	8.0
	M	1,600	60.0	50.0	8.0
Sephadex-Tyrosyl-tyrosine	C	65,000	9,000	5.2	6.6
	M	71,500	9,800	9.6	6.0
Calcium phosphate supernatant	C	52,000	55,000	1.6	4.6
	M	59,300	53,000	2.2	5.2
QAE-A50 Chromatography					
Peak I	C	19,000	300,000	0.26	1.1
Peak II	C	6,000	200,000	0.14	0.6
Peak I	M	16,000	290,000	0.25	1.0
Peak II	M	8,000	250,000	0.10	0.5

Cells (2×10^8) were grown for 48 h in normal medium (C) or medium containing MSH (M). The cells were collected, frozen and thawed, and homogenised in NaPO_4 (5 mM, pH 6.8). Homogenates were centrifuged at 20,000g for 30 min. Supernatants of the cells were then applied to Sephadex G-25 (superfine)-tyrosyl-tyrosine columns (8×25 cm) prepared by the method of Axen *et al.*⁹, and equilibrated in sodium phosphate (5 mM, pH 6.8). Tyrosinase was eluted from the affinity column with 0.3 M sodium phosphate (pH 6.8), concentrated with an Amicon apparatus, and dialysed against sodium phosphate (5 mM, pH 6.8). A calcium phosphate suspension (1 ml; 0.4 mg ml^{-1} ; Biorad) was added per mg protein. The mixtures were stirred at 4 °C overnight and centrifuged. Calcium

phosphate supernatants were then applied to QAE-A50 columns (2×25 cm) equilibrated with sodium phosphate (5 mM, pH 7.4). Tyrosinase was eluted with 0.05 M (peak I) and 0.10 M (peak II) sodium phosphate (pH 7.4). ^{14}C -labelled standard tyrosinase was prepared by the above procedure from cells grown for 48 h in medium containing MSH and ^{14}C -leucine (10 $\mu\text{Ci ml}^{-1}$; New England Nuclear). ^3H -labelled tyrosinase was prepared from cells grown in medium containing ^3H -leucine (100 $\mu\text{Ci ml}^{-1}$; New England Nuclear) with or without added MSH. Tyrosinase assays were by the method of Pomerantz⁸ in the presence of L-dopa (0.1 mg ml^{-1}). The above experiments were repeated three times with similar results each time.

not suppressed by either actinomycin D or cycloheximide (Table 1). As RNA and protein synthesis were inhibited these results suggest that neither is required for the MSH-mediated increase in tyrosinase activity. Results similar to these were obtained in ten out of twelve experiments. Note, however, that in two of the twelve experiments treatment of the cells with actinomycin D and MSH caused a 15 to 30-fold increase in tyrosinase activity over that in cells treated with MSH alone.

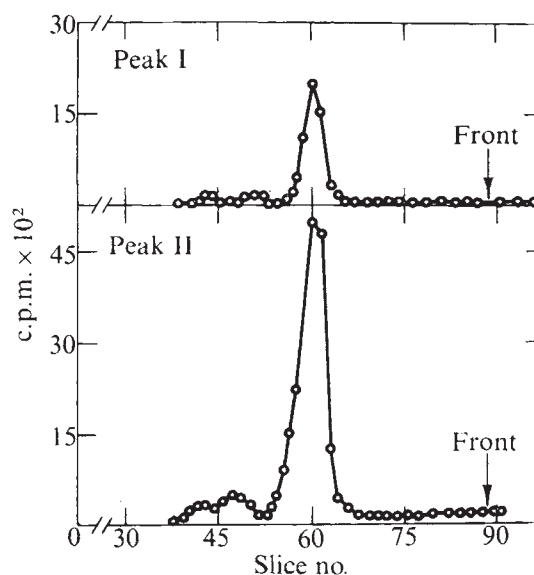
We next investigated whether MSH might act by preventing the decay of tyrosinase activity. Non-synchronised cells were grown for 48 h in normal medium or medium containing MSH. By that time tyrosinase activity in the MSH-treated cells had plateaued at a value which was five times that in the control cells. Protein synthesis was then blocked by cycloheximide, and tyrosinase activity was measured over an additional 24 h. The activity decayed with a half life of 12 h whether or not the cells were exposed to MSH (Fig. 1). This indicated that MSH did not alter the decay of tyrosinase activity in the cells.

We then tested for the presence of tyrosinase inhibitors in the cells. Cells were cultured for 48 h in normal medium or medium containing MSH. Crude extracts were prepared and tyrosinase activity was measured in the separate extracts and in 1:1 mixtures of extracts from control and MSH-treated cells. The results suggest that MSH promotes the inactivation of an inhibitor of tyrosinase function, as the only difference between the two extracts was previous exposure of the cells to MSH. Two additional findings suggested that tyrosinase inhibitors were present in the cells. First, when tyrosinase was partially purified by affinity chromatography on a Sephadex-tyrosyl-tyrosine column, the recovered activity was usually much greater than that applied to the column (Table 3). This was true both in control cells and those exposed to MSH, indicating that some inhibitors of tyrosinase still remained in the cells following treatment with MSH. Second, when partially purified tyrosinase was exposed to an aliquot of the crude extract from which it was isolated, the activity was greatly reduced (Table 2, d-e).

We developed a purification procedure for tyrosinase to compare the number of enzyme molecules in control cells and those exposed to MSH. Tyrosinase existed in both a soluble and particulate form in the cells. After exposure of the cells to MSH and partial purification of the enzyme, however, most of

the activity existed in the soluble fraction. For this reason, we purified the soluble form and the quantification results apply only to that form (Table 3). Two peaks of tyrosinase activity were obtained during a stepwise salt elution from a QAE-A50 column. These had identical mobilities when analysed by SDS-polyacrylamide gel electrophoresis (Fig. 2). Tyrosinase prepared from cells labelled with ^{14}C -leucine migrated through the gels in a major peak (molecular weight 32,000) containing more than 85% of the material and two minor peaks (58,000 and 66,000). The same electrophoretic patterns were observed when the gels were stained for protein with Commassie blue. The quantification procedure was as follows: cells were grown

Fig. 2 Electrophoretic analysis of ^{14}C -labelled tyrosinase from QAE-A50 peaks I and II. Tyrosinase was purified from cells labelled with ^{14}C -leucine (Table 3) and analysed on disc polyacrylamide gels (10%) containing SDS (0.1%). Electrophoresis was performed in Tris-glycine buffer (pH 8.8) at 5 mA per tube for 4 h. Gels were frozen and then sliced into 1 mm sections, which were digested overnight in H_2O_2 (30%) at 50 °C, and counted in Aquasol (New England Nuclear) in a liquid scintillation counter.



in the presence or absence of MSH for 48 h, during which time they were also exposed to ^3H -leucine. Extracts of the cells were then made, and equal amounts of ^{14}C -labelled standard tyrosinase which had been purified previously were added to each extract. Soluble tyrosinase was prepared and the ^3H -labelled enzymes were co-purified separately with the ^{14}C -labelled standards (Table 3). There were essentially the same number of ^3H counts in the purified enzymes whether or not the cells had been exposed to MSH. We conclude from these results that MSH did not promote the synthesis of new molecules of the soluble form of tyrosinase in the cells.

From the results presented above, we propose that the mode of action of MSH in causing increased tyrosinase activity in Cloudman S91 melanoma cells is to partially relieve an inhibition of the enzyme. Studies are under way to identify the molecular intermediates involved.

We thank M. Sansone, N. Koch, J. Morowitz, and T. Michaud for assistance, and A. Lerner for his interest and support. This work was supported by grants from the US Public Health Service and the American Cancer Society.

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Received March 6; accepted May 6, 1975.

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Partially single-stranded form of free Moloney viral DNA

SEVERAL hours after infection of cells by RNA tumour viruses new, free viral DNA molecules can be detected¹⁻³ which are apparently intermediate between the initial RNA-dependent DNA transcription product and the final integrated viral genome^{1,6}. Work on Rous sarcoma virus^{6,7} and Moloney murine leukaemia virus (Mo-MuLV)⁴ showed that this DNA is resolvable by ethidium bromide-caesium chloride (EB-CsCl) centrifugation into two classes of molecule. The first, from the lower band of the EB-CsCl gradient, contains closed-circular, double-stranded, supercoiled viral DNA molecules of molecular weight 5×10^6 – 6×10^6 , about twice the weight of the single-stranded subunits isolated from virion RNA. The kinetics of disappearance of these DNA molecules suggest that they are the precursors of viral genomes integrated into cellular DNA⁵. The second class of viral DNA, from the upper band of the gradient, is characterised here. These molecules have the properties of intermediates between the initially synthesised RNA-DNA hybrids and the double-stranded, supercoiled form of the genome. Careful preparation reveals that these molecules are partially single stranded. The first strand of the viral DNA is complexed with shorter segments of the subsequently synthesised second strand.

Two probes were used to measure the relative amounts of the two strands of viral DNA. ^{125}I -RNA, prepared by *in vitro* iodination of virion RNA (ref. 8), is complementary to the first strand of the proviral DNA synthesised on the parental RNA template. ^3H -cDNA (complementary DNA).

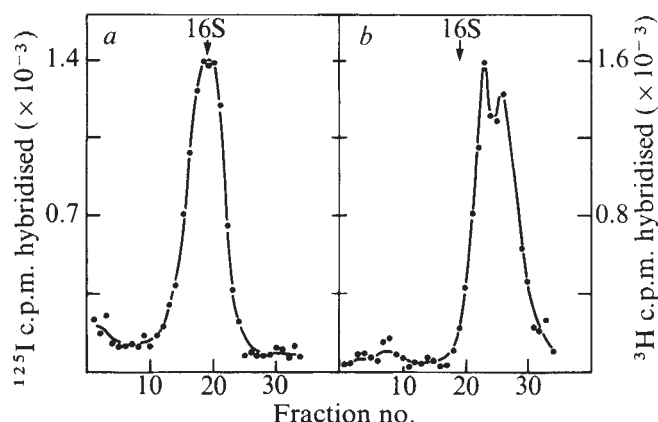


Fig. 1 Alkaline sucrose sedimentation profiles of proviral DNA. DNA was extracted by the Hirt procedure¹² from 2×10^8 JLS V-9 mouse bone marrow cells 4.5 h after infection by Moloney-MuLV at a multiplicity of 1 PFU per cell¹³. DNA was deproteinised and fractionated on an EB-CsCl gradient⁴. The material from the upper band was applied to two 15–30% (w/v) sucrose gradients made in 0.3 M NaOH, 0.7 M NaCl, 0.005 M EDTA, 0.1% Sarkosyl. Centrifugation was performed for 15 h at 36,000 r.p.m. at 20 °C in the Spinco SW41 rotor. Virus-specific sequences were detected by hybridisation with: *a*, 2,500 c.p.m. of viral ^{125}I -RNA (refs 4 and 8) or *b*, 2,500 c.p.m. of viral ^3H -cDNA (refs 4, 9 and 14). Sedimentation is shown from right to left. SV40 Form II DNA (native molecular weight 3.6×10^6) produced by cleavage with *EcoR*₁ endonuclease, was run on a parallel gradient as marker.

prepared by incubation of detergent-activated virions with radioactive nucleoside triphosphates⁸, is a probe for the subsequently synthesised second strand of the proviral DNA.

DNA was taken from the upper band of an EB-CsCl gradient and resolved by centrifugation through sucrose gradients under alkaline conditions. Fractions of the gradients were analysed for viral homologous DNA by hybridisation to each probe (Fig. 1). The $T^{1/2}$ profile of DNA complementary to ^{125}I -RNA indicates a distribution of molecules of molecular weight 8×10^5 to 3×10^6 (Fig. 1*a*). Most of these molecules of the first-strand DNA sedimented like molecules of about 1.5×10^6 daltons. Only the largest of these first-strand DNA molecules was equivalent in length to a complete transcript of the 35S virion RNA (3×10^6 daltons, about 21S). There was no suggestion of any circular molecules of complete genome length (23S).

The second strand of the proviral DNA was detected by its complementarity to ^3H -cDNA (Fig. 1*b*). This material was markedly smaller, sedimenting like molecules of about 5×10^5 daltons. The largest of these molecules had a molecular weight of about 9×10^5 . These second-strand DNA molecules were fragments of 1/30 to 1/3 the length of a complete transcript of the 35S virion RNA.

Further, there was almost no second-strand DNA (Fig. 1*b*) sedimenting at the rate of complete single-stranded transcripts of linear or circular conformation (20S, 23S). Therefore, the proviral DNA prepared from the upper band of an EB-CsCl isopycnic gradient was not derived from the supercoiled closed-circular DNA by endonucleolytic degradation of the supercoiled DNA during preparation.

DNA from the upper band of an EB-CsCl gradient was next hybridised to ^3H -cDNA or ^{125}I -RNA probes without further fractionation. Before hybridisation an aliquot of each preparation was mixed with radioactive probe and incubated under hybridising conditions while another aliquot was first denatured by boiling (Fig. 2*a*). Boiling before hybridisation increased by a factor of about 1.5 the number of sites available to hybridise to ^{125}I -RNA. Similar experiments have occasionally shown a smaller increase (20–30%) of available sites after denaturation. Together,

these experiments indicated that more than half of the first strand of the proviral DNA was available for hybridisation without previous denaturation. We concluded that this fraction of the first-strand DNA exists in the cell in a single-stranded form.

Hybridisation of proviral DNA to ^3H -cDNA without previous boiling resulted in a low level of hybridisation. Previous denaturation, however, caused a considerable increase in hybridisable viral DNA. Thus virtually the entire second strand of the undenatured proviral DNA exists in a double-stranded complex.

Interpretation of the previous experiments (Fig. 2) is complicated by possible artefacts deriving from displacement of resident strands of duplexed DNA by molecules of the probe¹⁰. Thus, annealing of ^{125}I -RNA to undenatured proviral DNA (Fig. 2a) could have been caused by the probe displacing some of the second-strand DNA from existing duplexes. These artefacts were ruled out by treating the undenatured proviral DNA with the single-strand specific nuclease S_1 .

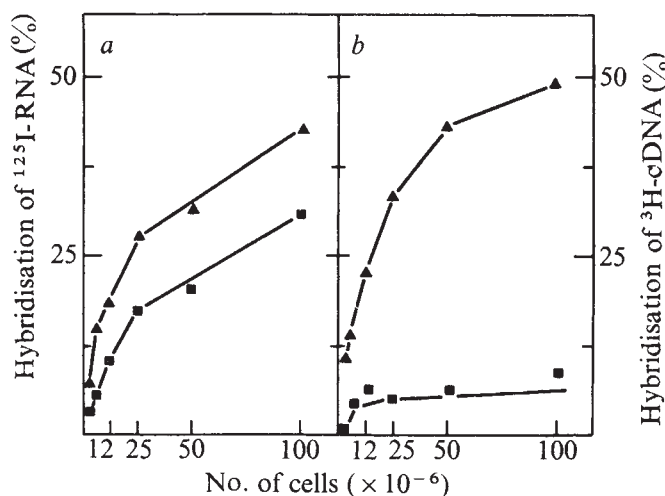


Fig. 2 Hybridisation of denatured (Δ) or undenatured ($\blacksquare$) virus-specific DNA with ^{125}I -RNA or ^3H -cDNA probes. Viral DNA was prepared from cells 9 h after infection following methods described for Fig. 1. Different sized samples of this DNA, comparable with extracts of the indicated number of cells, were hybridised to constant amounts (1,500 c.p.m.) of ^{125}I -RNA (a) or ^3H -cDNA (b).

Extracted proviral DNA was hybridised initially to radioactive probe with or without previous S_1 treatment (Fig. 3a). Incubation of the proviral DNA with the enzyme removed all first-strand DNA available for hybridisation to ^{125}I -RNA in the absence of denaturation. This confirmed that the first-strand DNA contained sites available for hybridisation by virtue of their single strandedness. Hybridisation to cDNA probe was low without previous denaturation (Fig. 3c) as before. Treatment with S_1 nuclease reduced this residual hybridisation of the second strand to ^3H -cDNA.

The experiments of Fig. 3a and c were controlled by parallel experiments on samples incubated in the presence or absence of S_1 nuclease, deproteinised, and then denatured before hybridisation. Figure 3b shows that about half of the total first-strand DNA was destroyed after S_1 nuclease treatment of undenatured DNA. In contrast enzyme treatment of the undenatured complex did not significantly reduce the amount of second-strand DNA available for hybridisation after subsequent denaturation (Fig. 3d). These experiments indicate that about half the first-strand DNA was initially found in a single-stranded state while the second strand DNA was virtually all in a double-stranded configuration.

These experiments suggested that proviral DNA of the first strand contained large amounts of single-stranded

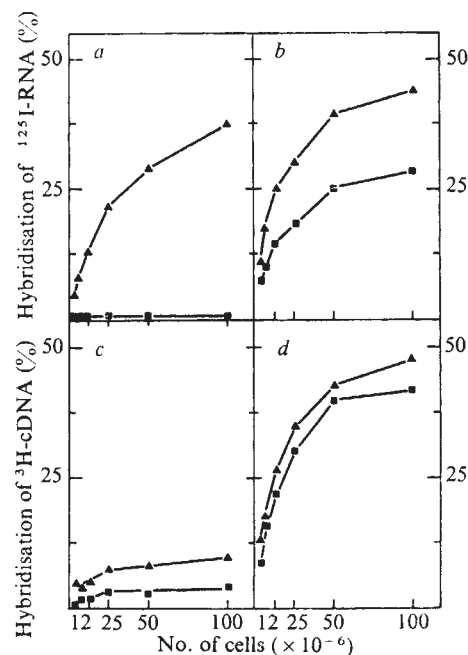
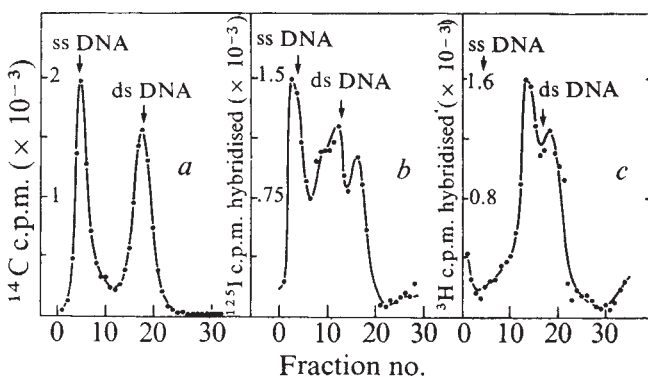


Fig. 3 Hybridisation of S_1 treated ($\blacksquare$) or untreated (Δ) virus-specific DNA with ^{125}I -RNA and ^3H -cDNA probes. DNA extracted 9 h after infection, prepared as in Fig. 1, was incubated in the presence or in the absence of S_1 nuclease¹⁴. Digestion conditions were chosen so that treated single-stranded DNA was no longer competent to form hybrids whereas the ability of double-stranded DNA to form hybrids after denaturation was unaffected. Increasing amounts of the DNA were annealed directly to probe (a and c) or after intervening denaturation (b and d). 1,500 c.p.m. of ^{125}I -RNA or ^3H -cDNA was used in all hybridisations.

DNA. This was confirmed further by isopycnic centrifugation of the complex on sodium iodide gradients, which separate single-stranded from double-stranded DNA (ref. 11 and Fig. 4a). The proviral DNA was banded and fractions were denatured and incubated with either of the probes. Hybridisation to ^{125}I -RNA (Fig. 4b) indicated that about one-fourth of the first-strand DNA was in purely single-stranded molecules while the remainder formed part of complexes with various degrees of double strandedness. The profile of the second-strand DNA (Fig. 4c) in the sodium iodide gradient showed that this DNA existed solely in

Fig. 4 Isopycnic centrifugation of virus-specific DNA in NaI gradients. Virus-specific DNA was prepared as in Figs 2 and 3 from 10^9 cells. NaI was added to a density of 1.546 g cm^{-3} and the resulting 10 ml solution was centrifuged at 45,000 r.p.m. for 70 h at 20°C for the Spinco 65 rotor¹¹. The gradients were denatured before hybridisation with probes. a, Mixture of native and denatured mouse DNA as marker. b, Profile of proviral DNA detected by hybridisation to ^{125}I -RNA probe or to c ^3H -cDNA probe.



structures which are largely or completely double stranded.

The proviral DNA complex studied here is present in several copies per cell from 4 to 10 h after infection. Its properties resemble an intermediate between the initial RNA-DNA hybrid and the final closed-circular, double-stranded DNA form. Little or no RNA is still associated with this complex, since treatment of the denatured form with alkali has no effect on the subsequent protection of ³H-cDNA probe (A. M. Gianni, unpublished).

Much of this DNA may not grow into complete genomes since the first strand of this complex is shorter than a complete transcript of 35S virion RNA and the second-strand DNA is present only in fragments covering only part of the first-strand molecules. An analogous Rous sarcoma viral DNA has recently been reported, containing complete first strand DNA fully covered by second strand fragments¹⁵.

This work was supported by grants from the American Cancer Society and the National Cancer Institute. R. A. W. is a Cancer Research Scholar of the American Cancer Society Massachusetts Division.

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Induction of a carcinogenic oncornavirus in C57BL/6 mouse embryo cells by 5-iododeoxyuridine

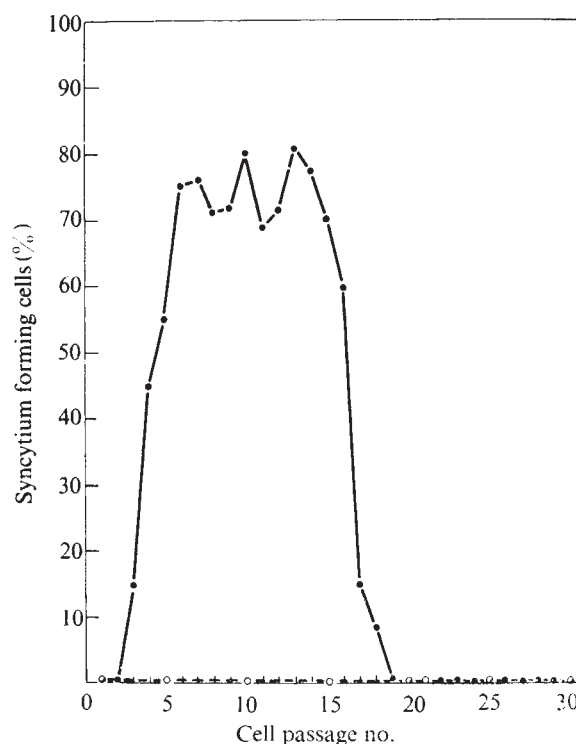
THE presence in mouse cells of oncornavirus-related genetic material has been documented¹⁻³ and the activation and release of latent oncornaviruses from virus-negative mouse cells by halogenated pyrimidines has been demonstrated⁴⁻⁶. The relationship, if any, between the endogenous viral genes, the activated oncornaviruses and the oncogenic process is not known, although one chemically activated mouse oncornavirus was shown to induce lymphatic leukaemia⁷. Here we describe a system in which the induction of an oncornavirus is followed by the transformation of an epithelioid line of mouse embryo cells to carcinoma cells. This system is particularly interesting because the normal cells are derived from a mouse strain with a very low incidence of spontaneous cancers⁸, and also because the majority of tumours in man arise from epithelial tissues^{9,10}.

Cell cultures were initiated from pooled whole embryos of individual C57BL/6 mice at 12-14 d gestation. Growth medium consisted of RPMI 1640 supplemented by 10% heat-inactivated foetal calf serum, and cultures were transferred twice weekly. A total of 17 such cultures was isolated, of which five developed into cell lines and one

of these, M6, was chosen for induction experiments. This cell line was of epithelioid type.

M6 cells at passage 31 were used for induction. 5-Iododeoxyuridine (IdU) has been shown to be an efficient inducing agent of oncornaviruses in mouse cells⁴⁻⁶, and the steroid dexamethasone (DM) is capable of enhancing the production of type-C virus induced by IdU from cultured mouse fibroblasts¹¹. M6 cultures were treated with IdU+DM as described in legend to Fig. 1 and examined for virus production by the reverse XC test¹², modified as described in the legend to Fig. 2. This test has been shown to detect murine leukaemia virus-producing cells^{12,18}. Three to four days after IdU treatment the cultures began to show a toxic effect, from which they recovered and regrew to form confluent cell monolayers. During the whole of the period before the first cell passage, XC tests were negative. In other induction systems examined virus was transiently detected within the first few days of addition of inducer^{7,11}. Figure 1 shows the percentage of syncytium-forming cells (SFC) obtained per culture as a function of cell passage after addition of inducer. In this experiment the number of SFC in the first and second passage was 0 and 0.01%. Thereafter, the SFC rapidly increased to about 75%. This level was maintained for a further nine cell passages after which it declined to levels below 1% (M6V⁻ cells). The reason for this decline is being investigated. In two additional repeat experiments, in which M6 cells at passages 37 and 45 were used, similar kinetics of syncytium production was observed but with some variation in the time of appearance of syncytia. It was noted that the high level of syncytium-forming cells was maintained for the same number of cell passages in the three experiments indicating that this process is strictly regulated. Passaged and non-treated M6 cells were negative in the

Fig. 1 Induction of M6 cells. 1×10^6 cells in growth medium were plated in 100-mm Petri dishes. Twenty-four hours after plating $40 \mu\text{g ml}^{-1}$ IdU (5-iodo-2'-deoxyuridine, Sigma) was added. After a further 24 h, the cultures were washed three times with 10 ml RPMI and fresh growth medium containing 10^{-8} M dexamethasone sodium phosphate (MSD, Decadron) was added. Virus induction was measured by a modified reverse XC test¹² as described in legend to Fig. 2 and syncytia were counted microscopically. ●, Treated cells; ○, untreated cells.



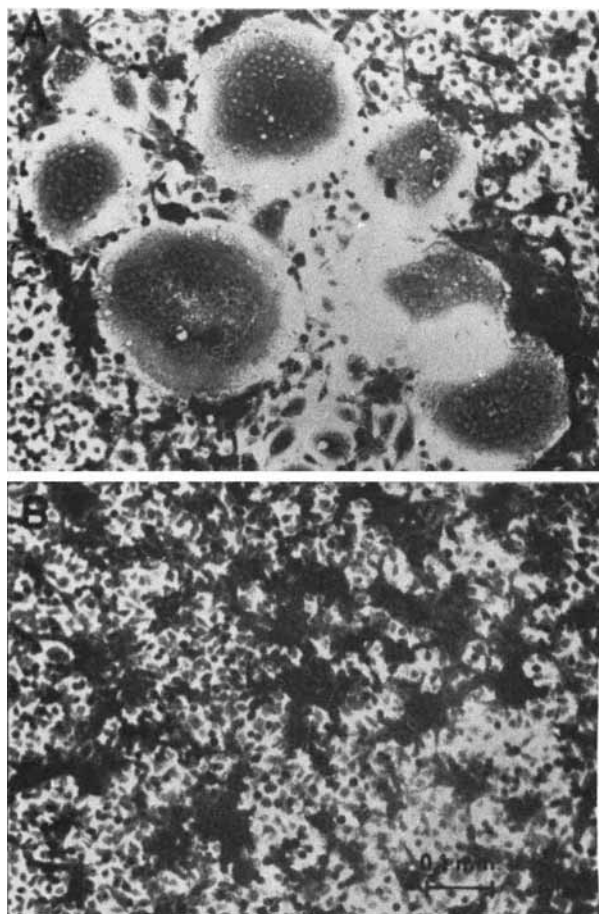


Fig. 2 Syncytia formation by virus-producing cells. Test cells 10^4 were cocultivated with 10^6 XC cells in 5 ml growth medium in 50 mm Petri dishes. On day 4 the cultures were fixed and stained in methanol-gentian violet solution and syncytia were counted microscopically. Syncytia with less than five nuclei were not included. A, Induced or BVI-infected M6 cells; B, untreated M6 cells.

XC test (Figs 1 and 2). The data suggest that M6 cells were activated by IdU to produce an oncornavirus (BVI). The number of cells initially activated may be very low and the high plateau levels of syncytia reached in further cell passages may be the result of horizontal infection of uninduced cells by the endogenous virus and/or preferential replication of virus-producing cells, rather than to the progressive expression of the induced state.

The possibility of horizontal infection and virus replication in M6 cultures was examined. Supernatant fluid from BVI-producing cultures was used as a source of virus to infect M6 cells¹⁴, and the cells were tested by the XC test. A high level of cells scored as SFC on the second passage after infection, and this level has now been maintained for more than sixty cell passages. Supernatants from induced cultures can therefore infect M6 cells, replicate in them and establish a chronic virus-producing cell line (M6BVI). The ability to infect and replicate in homologous cells classifies this endogenous oncornavirus as ecotropic¹³, although this property may be attributable to selection.

Oncornaviruses can also be detected by their characteristic density in sucrose. Induced and control M6 cultures were labelled with ^3H -uridine and the results show that supernatant fluids from induced, but not control, M6 cultures yielded a peak of radioactivity at a density of 1.16 g cm^{-3} . This indicates that BVI is a particle with a density characteristic of oncornaviruses and is released from cells.

Electron microscopic examination of thin sections of

induced M6 cells showed C-type particles in the intercellular spaces (Fig. 3A). Two stages of budding particles were seen on the periphery of some cells (Fig. 3B, 3C). Such particles have not been seen in uninduced M6 cells. Negative staining of sucrose-banded BVI virus showed an electrondense core surrounded by a core shell and an outer envelope. The particles have a diameter of 1,000–1,400 Å and seem to be of uniform shape (Fig. 3D).

Although M6V⁻ cells failed to produce syncytia (Fig. 1), indicating the lack of production of BVI virus, this does not exclude the possibility that these cells produce some other oncornavirus type. To test this, M6V⁻ cultures were labelled with ^3H -uridine. No radioactivity was detected at a density characteristic of oncornaviruses. The further possibility that M6V⁻ cells contain a partially activated oncornavirus can also not be excluded from these data.

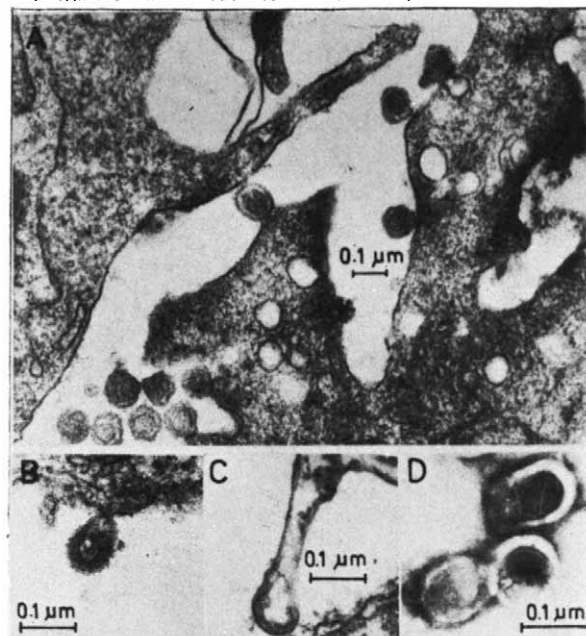
It was of interest also to examine other properties of these cells and to compare them with those of the uninduced M6 cells. The properties examined were infectivity by BVI and another oncornavirus, RadLV¹⁵, reinduction by IdU and growth behaviour of cells *in vitro* and *in vivo*. It should be noted that the uninduced and the induced cells were at the same cell-passage level when tested.

M6 and M6V⁻ cultures were infected with BVI or RadLV and tested by the XC test for up to four cell passages post infection. Whereas the M6 cells were competent to replicate both viruses, M6V⁻ cells were resistant.

M6V⁻ cultures were also tested for their behaviour towards IdU. M6V⁻ cultures were treated with IdU and dexamethasone as described in legend to Fig. 1 and tested by the XC test. M6V⁻ cells gave no syncytia even after ten cell passages after addition of IdU, suggesting that these cells differ from the untreated M6 cells.

A further contrasting feature of these cultures was their growth behaviour *in vitro*. When single cells of both types were seeded on Petri dishes M6V⁻ cells formed macroscopic colonies at a rate of 10^{-2} although no colonies formed from 10^4 M6 cells. Again, when these two cell types were seeded in soft agar¹⁷, M6V⁻ cells produced 30 times as many colonies as M6 cells.

Fig. 3 Electron microscopy of induced M6 cells and BVI particles. For thin sectioning small pellets of induced M6 cells were prepared according to the rapid fixation technique described by Hayat and Giaquinta¹⁶. For negative staining, viral pellets were stained with 2% aqueous uranyl acetate. All preparations were examined in a Philips 300 electron microscope. A, B and C, Thin sections of induced virus-positive M6 cells; D, negative stain of sucrose-banded and pelleted BVI virus.



The above-mentioned cell-growth patterns indicated that M6V⁻ cells are transformed non-producer cells. To test this, 1×10^6 cells were inoculated subcutaneously into adult syngeneic mice and these were observed for tumour production. The results are shown in Fig. 4. Whereas 1 out of 46 mice inoculated with M6 cells eventually produced a small growth, 17 out of 18 mice inoculated with M6V⁻ cells developed large tumours at the inoculation site. The tumours first became palpable on day 23 after inoculation and grew progressively larger. By day 42, 95% of the mice had tumours. Mice of both sexes were equally susceptible to tumour formation. An additional group of 28 mice was inoculated with BVI-infected cells. Of these mice 96% developed large tumours by day 47 (Fig. 4). One of these tumours has been recultured and shown to be virus positive by the XC test. Histological examination of tumour sections revealed that these were carcinomas.

The system described here has the following salient features. (1) The cells originate from normal mouse embryos derived from a mouse strain with a very low incidence of spontaneous tumours. (2) The cell line used for virus induction is epithelioid. (3) The cells are regularly inducible. (4) They yield an endogenous oncornavirus. (5) The virus is ecotropic. (6) Induced and infected cells become transformed *in vitro* and produce carcinomas in syngeneic mice.

The data indicate that the induced cells as well as the infected cells are transformed and furthermore suggest that although virus production is not required for the maintenance of cell transformation (M6V⁻ cells), some viral function(s) is probably needed for its initiation.

Whether more than one virus is induced has not yet been

established. IdU may induce only one endogenous virus which is immediately oncogenic or could acquire oncogenic capacity during replication and/or integration at a different site in the cell genome. This virus could also be the inducer of a second virus with oncogenic properties. Alternatively, IdU may induce two viruses simultaneously, one oncogenic and the other its helper. In M6BVI cells these viruses may coexist while in M6V⁻ cells the helper is suppressed.

Since we have obtained carcinomas, this system may be a useful model to investigate the commonest type of neoplasia in man¹⁰. Up till now only those tumour viruses which induce tumours of mesodermal cells, the sarcomas and leukaemias, have been studied *in vitro*.

We thank Professor Natan Goldblum for support and interest, Dr M. Kotler for providing a seed of XC cells, Dr M. Haas for a breeding nucleus of C57BL/6 mice and Professor M. Sachs for examination of tumour sections. This work was partially supported by the Judith Segal Fund.

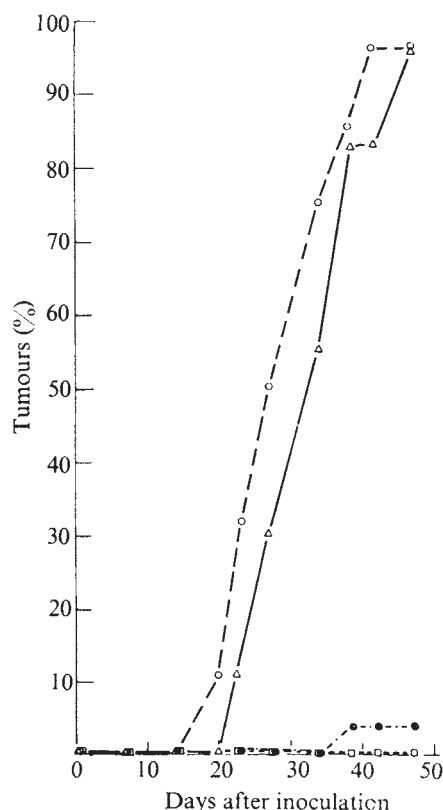
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Fig. 4 Tumour formation. 1×10^6 cells were inoculated subcutaneously on the back of adult C57BL/6 mice and observed for tumours. Number of mice inoculated is indicated in parentheses. M6/28 and M6/98 were early and late-passage M6 cells respectively. M6BVI and M6V⁻ cells were at the late passage (P98) level. Mice were scored as tumour positive when palpable growths first appeared. The data represent the cumulative tumour incidence. ○, M6BVI(28); △, M6V⁻(18); ●, M6/98 (28); □, M6/28(18).



Enzyme thermostability is a transformable property between *Bacillus* spp.

WE are interested in the evolutionary adaptation of enzymes and their control systems in different environments. We have studied the thermostability of organisms and their enzymes with DNA-mediated transformation between *Bacillus* spp. using an essential biosynthetic enzyme as a well-defined biochemical marker. Purification and characterisation of the enzyme L-histidinol dehydrogenase (HDH), EC 1.1.1.23, from a range of temperature-adapted species^{1,2} has shown that although they possess similar physical characteristics, Michaelis constants, molecular weights, subunit numbers and pH optima, they show vastly different temperature ranges of activity. Singleton and Amelunxen³ conclude that present evidence is incompatible with any single theory of the mechanism of thermophily. There seems to be a general finding, however, that the amino acid sequence itself confers thermostability, either through disulphide bridge interaction, by an increased proportion of hydrophobic amino acids, or by an interaction with metallic ions giving an altered conformation.

Recent results demonstrated genetically definable com-

ponents involved in temperature adaptation. Howard³ found a single gene which allowed growth at 1 °C in *Pseudomonas* and McDonald⁴ found a single gene conferring growth at 55 °C on *B. subtilis*. Both these genes were found to be closely linked to the streptomycin resistance locus, indicating some possible ribosomal involvement—as speculated by McDonald. Furthermore, it was found⁵ that organisms failed to grow at high temperatures unless 5-methyl-2-thiouridine had replaced ribothymidine in the tRNA sequence GT ψ C; otherwise this region fails to interact with 5S RNA in 50S ribosomes at such temperatures.

We have found that DNA from a thermophilic bacillus can transform a mesophilic strain of *B. subtilis* so that it will grow at high (>70 °C) temperatures. Cotransference of genes mapping close to those coding for ribosomal and tRNA functions were observed. Examination of the temperature activity characteristics of HDH showed that the transformed cells possess a thermostable enzyme similar to that present in the DNA donor cells. We suggest that, in this case, a mechanism for thermophily exists as a result of specifically altered translation which produces modified proteins with increased thermostability.

Two organisms were used, *B. subtilis* 25 hisB (31) (*str^s purA⁻* (*ade* 16) *leu⁻ his⁻ trp⁻ met⁻*; growth optimum 37 °C) as recipient and *B. caldolyticus* (*str^r purA⁺* (*ade* 16) growth optimum 72 °C) as donor. DNA was extracted from *B. caldolyticus* and purified by treating the cells with 100 μ g lysozyme in 5 ml 0.01 M EDTA (pH 8.0) for 15 min at 37 °C; 10 μ g Pronase was added and the cells stored at 4 °C for 16 h; the resulting lysate was centrifuged to equilibrium in an ethidium bromide-caesium chloride gradient and the DNA band isolated⁶.

Transformation was carried out by the method of Spizizen⁷. Competent *B. subtilis* cells were treated with DNA (2.5 μ g ml⁻¹) for two periods (40 min and 4 h) after which 50 μ g ml⁻¹ DNase was added. Transformants for streptomycin resistance, and for adenine, histidine, leucine, tryptophan and methionine prototrophy were selected at 37 °C after growth of the treated cells in a rich broth for 4 h to enable expression. Selection for high temperature growth ability was made by allowing samples of the cells, treated for 4 h and grown at 37 °C, to grow further in a defined liquid medium for thermophiles at 70 °C for 18 h and then plating for single colonies at 55 °C (Table 1).

Transformants inheriting streptomycin resistance or adenine prototrophy were obtained from the thermophilic donor at 37 °C, and selection at high temperature yielded transformants capable of growth at 70 °C. No transformants arose when selection was made for inheritance of histidine, leucine, tryptophan, or methionine prototrophy from the donor. Work^{8,9} on DNA-mediated transformation between *Bacillus* spp. particularly on antibiotic resistance markers which, in most cases, are known to be associated with ribosomal proteins¹⁰, has shown that there is a gradient of conservation of the DNA sequences coding for purine (*ade* 16, close to the origin of

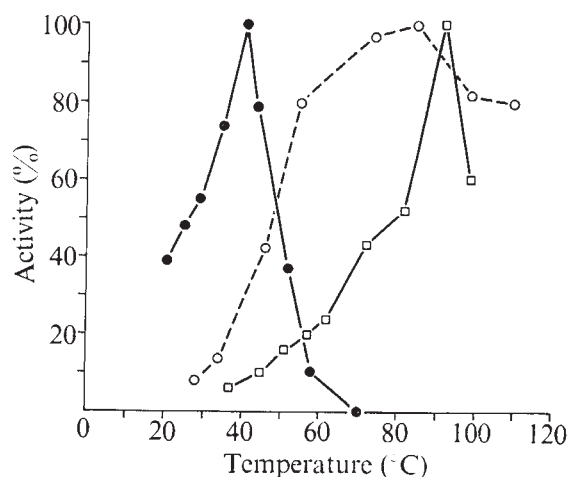


Fig. 1 Effect of temperature on HDH activity. ○, *B. caldolyticus*; ●, *B. subtilis*; □, transformant.

replication), ribosomal and tRNA functions. The conservation of these regions has been attributed to the possibility that modification of them may be less well tolerated than in other regions of the genome¹¹. It would seem reasonable to consider that temperature adaptation might be a manifestation of the action of modified genes in this region of the chromosome.

Further analysis of the transformants revealed markedly different growth rates at different temperatures. At 70 °C, some 20% of the transformants grew faster than the donor, and 10% grew much more slowly; at 37 °C these ratios were similar. These data suggest that more than one gene confers high temperature growth ability. For complete thermostability a majority of these genes must be transferred, varying growth rates being determined by the number of high temperature genes inherited.

One transformant (HT-1) was selected for examination of its HDH activity and the results obtained are probably the most important finding of this work. Whereas the original *B. subtilis* HDH was inactive above 70 °C, the HDH from HT-1 was thermostable even at 100 °C. Figure 1 shows the activity curves at various temperatures for the purified HDH enzymes from the three organisms tested. Identical results were obtained whether the enzyme preparation was in a crude extract or as a pure protein. The HDH from HT-1, however, was not identical with that from *B. caldolyticus* in that it required 2-mercaptoethanol for full activity; this may indicate some change in tertiary structure. We are currently sequencing HDH from a range of thermally adapted *Bacillus* spp. to elucidate any amino acid changes which may be a basis for increased thermostability.

Alternative interpretations of our results may be considered.

Table 1 Transformation for high temperature growth

	Mesophilic plates (37 °C growth)			Thermophilic plates (70 °C growth)	
	Supplemented	-Adenine	+ Streptomycin	Standard	+ Streptomycin
Control I: no DNA, no bacteria	0	0	0	0	0
Control II: DNA, no bacteria	0	0	0	0	0
Control III: + bacteria, no DNA	about 10 ⁹	0	0	0	0
Control IV: DNase from time 0, + bacteria, DNA	about 10 ⁹	0	0	0	0
DNA treatment for 40 min	about 10 ⁹	231	492	714	11
DNA treatment for 4 h	about 10 ⁹	308	512	1,040	203

Standard mesophilic plates: Spizizen salts, 0.5% glucose, 1% casamino acids, + 20 μ g ml⁻¹ leucine, histidine, tryptophan, methionine, adenine. Standard thermophilic plates: salts, 1% glucose, brain heart infusion, broth (McElroy)¹⁰. Salts per litre distilled water: NH₄NO₃, 0.5 g; KH₂PO₄, 0.25 g; MgSO₄, 0.25 g; NaCl, 0.025 g; FeSO₄·7H₂O, 0.05 g; CaCl₂, 0.05 g. pH adjusted to 7.8 with Na₂SiO₃; trace elements, 0.15 ml. Trace elements per 100 ml distilled water: MnCl₂·H₂O, 2.2 g; ZnSO₄·7H₂O, 0.05 g; H₃BO₃, 0.5 g; CuSO₄, 0.016 g; Na₂MoO₄·2H₂O, 0.025 g; CoNO₃·6H₂O, 0.046 g; H₂SO₄ (conc.), 0.5 ml.

Sufficient individual genes, each coding for a single enzyme, may have been transferred to enable the recipient to grow at high temperatures, or a small number of genes controlling wide pleiomorphic effects may have been transformed. The probability of multiple transformations of the required frequency is so low that we strongly favour the second alternative. A plausible hypothesis is that changes in the protein synthesis ability of thermophiles at the ribosomal or tRNA level give rise to translationally altered enzymes which can function at high temperatures. Such change in the translation machinery, as a result of the action of 'thermal adaptation' genes, would be expected to modify a large number of enzymes and other proteins in the transformed cell. Singleton and Amelunxen point out that there often seems to be an increased level of hydrophobic amino acids in proteins from thermophilic sources compared with their counterparts in mesophiles. It would be expected that a transformable mechanism of this nature would be of wide importance for the production of bacterial enzymes with increased resistance to high temperature and possibly to other extremes of the environment.

We thank Professor W. Hayes, Professor E. Elizur and Dr B. Rolfe for discussions, and Professor Elizur and Dr R. McElroy for bacterial strains.

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Received February 17; accepted May 1, 1975.

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Detection of informational complementarity in an amino acid sequence

THE existence of extensive base-paired secondary structure in a messenger RNA (mRNA) is of considerable importance in the analysis of the evolution and biosynthesis of the protein thus encoded¹. The amino acid sequence encoded by a looped mRNA sequence consists of two halves that are informationally related, but detection of such relationships in naturally occurring amino acid sequences is complicated by the three possibilities in phasing codons in the antiparallel RNA sequences of the loop. The triplet nucleotide codons within such a loop can be mismatched in two ways or can be exactly matched. Mathematical analysis of naturally occurring amino acid sequences to detect regions of possible informational complementarity is considerably simplified if one restricts the search to situations in which codon to codon matching in RNA loops is possible.

A simple computational scheme, based on the restriction of codon to codon matching, has been devised to detect the presence of informational symmetries in amino acid sequences. The computational scheme is described in detail below as applied to the tetradecapeptide somatostatin (somatotropin release inhibiting factor, SRIF). This peptide has been sequenced² and the sequence confirmed by synthesis³. The amino acid sequence is shown at the top of the matrix in Fig. 1a. The amino acids are numbered from the N terminal and the first line of the matrix lists the amino acids whose codons

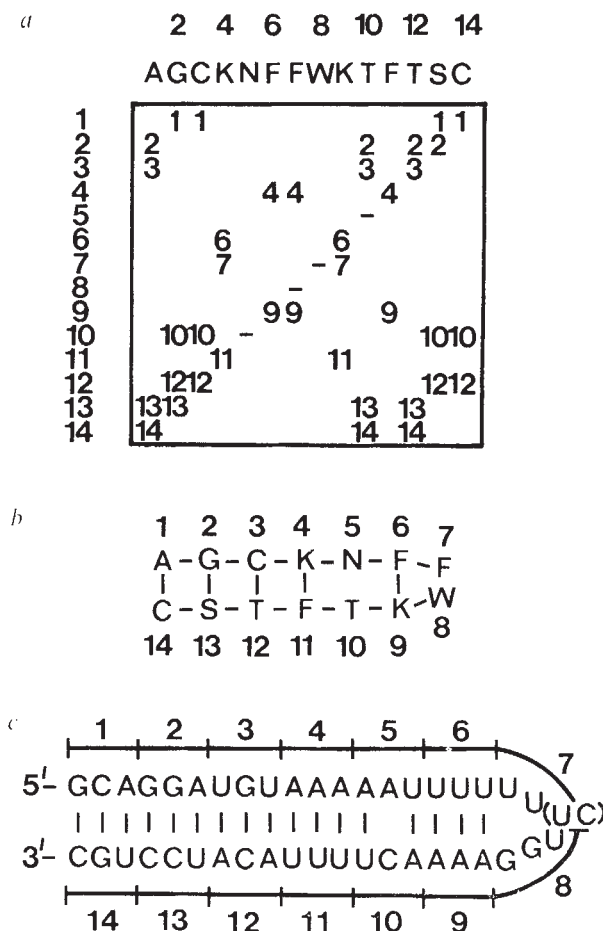
could be complementary to codons for the first amino acid (alanine) in a looped mRNA molecule, thus:

	Ala	Ala	Ala	Ala
5' -	GCU -	GCC -	GCA -	GCG . . .
3' -	CGA -	CGG -	CGU -	CGC . . .
	Ser	Gly	Cys	Arg

The 14 by 14 matrix was completed as shown in Fig. 1a and examined for maximum length sequences of possible codon complementarity. Tinoco *et al.*⁴ have used the same method, but including G-U base-pairing, to detect secondary structure in known RNA sequences. The maximum sequence is clearly defined by the bottom left to top right diagonal of the matrix. Note that the construction of a possible RNA loop is restricted by the requirement for unpaired nucleotides in the head of the loop. The optimal arrangement of codons is shown in Fig. 1b. The amino acid sequences of each half of the molecule are largely informationally related so that an hypothetical mRNA (or DNA) molecule, coding for somatostatin and exhibiting near perfect base pairing, can be constructed from the sequence (Fig. 1c). The invariance of the middle nucleotide of the genetic code for each amino acid (except serine) and the location of maximum variability in the 3'-terminal nucleotide position of codons allow more possibilities for theoretical maximisation of nucleotide base pairing in a codon to codon matching arrangement than in mismatched arrangements. It is remarkable that the minimum-energy RNA molecule falls short of 'perfect' complementarity by several atoms—a 4-amino instead of a 4-keto in the unpaired C of codon 10 (Fig. 1c).

The general matching scheme shown in Fig. 1b indicates a remarkable symmetry about an axis through the middle of the

Fig. 1 Informational complementarity of amino acid sequences in somatostatin. a and b, Letters represent amino acids (see ref. 6); c, letters represent ribonucleotides.



molecule. The appropriate criterion for estimating the significance of this degree of informational symmetry is the probability (P) that a random amino acid sequence of the same length would have an equal or greater degree of symmetry and not merely the probability of the occurrence of this degree of symmetry in a random sequence of the same length⁵. Assuming a value of four complementary amino acids per amino acid (this is in fact the maximum possible value), $P = 0.001$ for the symmetrical arrangement found for somatostatin. If one assumes an average value of three complementary amino acids per amino acid, $P = 0.0003$.

This computational scheme can be used to determine those amino acid sequences for which it is possible to construct theoretical looped mRNA sequences involving extensive codon to codon matching. Note that although the computational scheme will detect such mRNA loops if they actually exist, the scheme will only detect such loops as theoretical possibilities. Application of this computational scheme provides a novel way of examining the primary structure of polypeptides. This may be of considerable use in the study of the evolution and functional significance of amino acid sequences.

I thank Drs C. J. Thompson and M. J. Denton for discussions. This work was supported by the Australian Research Grants Committee.

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Table 1 Totals and merging residuals for data sets

	Centric data	Acentric data	Anomalous pairs	R_m (%)	R_d (%)
Native apoferritin	354	468	—	5.0	—
PCMB derivative	354	466	445	4.2	23.1
Uranyl derivative	354	468	452	6.3	28.0

$$R_m, \text{ merging residual} = \left(\sum_n \sum_{i=1 \rightarrow n} |F_{h,i} - \bar{F}_h| \right) / \sum_n n \bar{F}_h$$

$$R_d, \text{ difference residual} = \left(\sum |kF_{PH} - F_P| \right) / \sum F_P$$

after scaling to protein data.

with thioglycolic acid at pH 4.3 and a mercury derivative by further reaction with PCMB. Crystals of native apoferritin and of the mercury derivative were grown in 1–2% 3CdSO₄·8H₂O, and transferred to 1% cadmium sulphate solution before photography. For the second derivative, native crystals were soaked for 3 d and photographed in a solution of 1% cadmium sulphate, 1 mM UO₂(NO₃)₂, and 0.1 M acetate buffer pH 4.0. Long soaking led to deep coloration and crystal splitting, whereas high pH values gave large differences in intensities and deterioration to a low angle pattern.

Crystals were mounted for photography in quartz capillaries and precession data were collected with Cu K α radiation on a Philips 1120 generator with Elliot fine-focus tubes at 40 kV/30 mA. Exposures were made using 2- or 3-film packs for up to 2 or 3 d, a period in which radiation damage was not extensive. Intensities were measured with a Joyce–Loebl microdensitometer: no absorption correction was applied.

The 6-Å data set contains 824 reflections of which 354 are centric. The high symmetry ensures that most reflections were multiply determined, including most of the anomalous pairs. The data sets used are shown in Table 1. Film packs were scaled together by the method of Monahan *et al.*¹⁰

The structure of the PCMB derivative was solved by comparison of normal and anomalous Patterson functions: one mercury atom was located directly and a second was found in the phased error map and confirmed in the Pattersons. A reasonable electron density map of the protein was obtained at this point and, when anomalous scattering data was included, a clear choice of absolute configuration could be made by comparing alternative protein maps using such criteria as levelness and emptiness of the evident solvent regions. Difficulties were experienced in locating uranyl groups from Patterson maps and their positions were only found when heavy atom difference Fourier maps were calculated using phases obtained from the PCMB derivative. Although the uranyl derivative is inferior to that of PCMB, the combined data (including anomalous¹¹ differences from both derivatives) give a better electron density map, as judged by protein features as well as solvent flatness. Atomic parameters were refined by full matrix least-squares against the centric data for each derivative (Table 2).

The solutions include negative cadmium atoms corresponding to cadmium of crystallisation in the native structures (see also discussion below). These gave refinement problems as they tended to move towards nearby positive heavy atoms. Accordingly some of the positional parameters in Table 2 have been arbitrarily fixed, as well as the temperature factors.

The protein structure (connected electron density) fills about half the crystal cell and contains dense straight rods and sharp peaks (see Fig. 1) as well as irregular regions. It forms a hollow shell (centred on lattice points of 432 symmetry) with internal and external radii 35–40 Å and 62–65 Å respectively. This agrees with measurements of 37 Å/61 Å from low angle X-ray scattering in solution¹² and low angle crystal data⁶. The shell has six channels through it along the molecular fourfold axes. The channels are square in cross section 9–12 Å across, widening towards the inside, and might enable flat molecules up to 15 Å wide to penetrate the shell.

The first surprise in the structure was the appearance of two peaks in the map 50% higher than any other feature. These

Structure of horse-spleen apoferritin at 6 Å resolution

FERRITIN is the principal iron storage molecule in a wide variety of organisms¹. It consists of a hollow protein shell (apoferritin) which can contain up to its own weight of hydrous ferric oxide-phosphate as a microcrystalline micelle. Both the shell and its contents have been observed in electron micrographs^{2,3}. Ferritin and apoferritin crystallise in a number of forms, including a cubic one in which the round molecules are “close-packed”⁴. The cubic crystals have space group F432 (No. 209), side $a = 184$ Å, and contain four molecules per unit cell⁴. We report here on the electron density map of cubic horse-spleen apoferritin at 6-Å resolution which we have obtained using two isomorphous derivatives, formed with parachloro-mercury benzoic acid (PCMB) and uranyl nitrate.

It was originally proposed⁴ that apoferritin (molecular weight 445,000)^{1,5} contained 24 subunits, corresponding to the lattice symmetry of the cubic crystal form. Then on the basis of early subunit weight determinations, and apparent “spikes” in the X-ray diffraction patterns (by analogy with icosahedral virus particles recently characterised at the time), a 20-subunit model was proposed⁶. Now the wheel has come full circle; recent subunit weight estimates of 18,500–19,000 (refs 1, 5 and 7) agree well with a 24-subunit model, and application of the rotation function⁸ to the cubic crystal data at 9 Å has not shown the presence of the non-crystallographic symmetry which a 20-subunit model would require⁹. Finally, the solution of the cubic structure reported here in itself confirms the 24-subunit model. Each molecule lies on a lattice site of octahedral symmetry (with 24 equivalent positions), and we can see enough detail to reassure ourselves that the molecular and site symmetry are indeed identical.

Apoferritin was prepared by reducing commercial ferritin

we interpreted as cadmium ions of crystallisation. One is in the intermolecular contact area close to the twofold axis. This is evidently a salt bridge and explains the specific requirement of a divalent metal ion for crystallisation. The other is in the protein shell, on or near the threefold axis, at a radius of 54 Å. Our interpretation of these peaks has been confirmed in heavy atom error maps calculated using only mercury or uranyl atom solutions. In each case large negative peaks appeared corresponding to positive peaks in the protein map. These are therefore atoms in the native structure displaced in the isomorphous derivative; we thereafter included "negative cadmium" ions in the heavy atom solutions and refined their parameters. PCMB and uranyl nitrate remove the peak on the threefold axis: trial refinements suggested that three symmetry-related atoms contribute to this peak. Uranyl in addition displaces the other cadmium as an intermolecular salt bridge: it lies on the twofold axis between the two original cadmium sites.

The dense rods in the structure correspond in length to 50–60% of the protein being α -helical, in agreement with estimates from ORD measurements^{13,14} and a prediction of 55% from amino acid composition based on the formulae of Krigbaum and Knutton¹⁵ which we have made using the data of Williams and Harrison¹⁶. Two nearly-parallel rods, 35 Å and 40 Å long, are close to and normal to a twofold axis. They are 7 Å apart at their nearest point and assuming they are α -helical we must predict two regions of sequence each containing three or four glycines occurring every third or fourth residue to allow such a close approach. The twofold axis lies in a broad interface defined by two pairs of these helices. Their closest approach across the axis is 11–12 Å. A third long rod lies on the outer surface between the two- and threefold axes. The fourfold channels lie between roughly parallel rods which loop back into the rest of the structure at the outer end. Dense material around the threefold axis includes the bound cadmium ion: it may contain short helices and possibly more bound ions. The irregular regions of electron density which are also visible are not sufficiently well defined to allow us to trace out a complete subunit chain.

The binding sites revealed in isomorphous derivatives (of which apoferritin forms many) are interesting for two reasons. First, we may locate amino acid positions even before the full sequence and connectivity are known. Thus PCMB binds covalently at two sites: one is near the displaced cadmium

Fig. 1 A spherical shell model of apoferritin, cut away to expose dense regions from the electron density map. Twofold ($\parallel$), threefold (Δ) and fourfold ($\perp$) molecular symmetry axes are shown. Note the channels along the fourfold axes, the pairs of helices related by twofold axes (bottom), and peaks attributed to cadmium of crystallisation ($\bullet$).

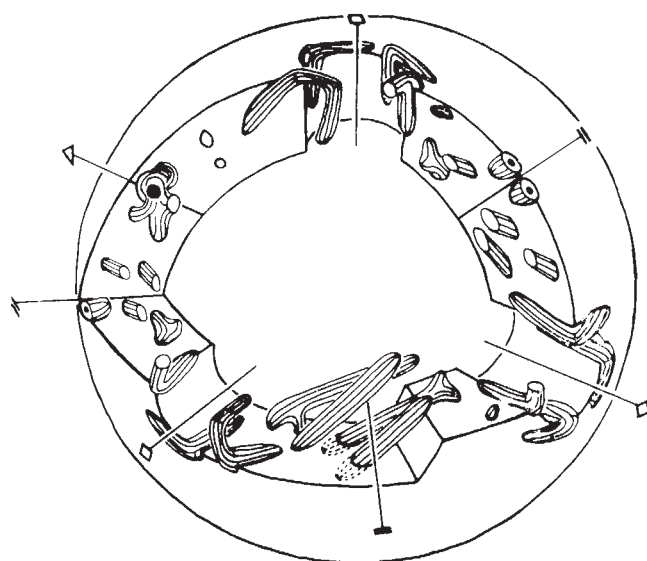


Table 2 Heavy atom parameters after refinement

Derivative	Atom	Occ.*	x/a	y/b	z/c	Radius (Å) [†]	U_{iso} (Å ²)
PCMB ($R_c = 36\%$) [‡]	Hg	0.27	-0.105	0.025	0.209	43.3	0.8
	Hg	0.39	-0.179	0.162	0.198	57.4	1.0§
	Cd	-0.20	-0.164¶	0.164¶	0.179	54.0	1.0§
Uranyl nitrate ($R_c = 47\%$) [‡]	UO ₂	0.32	0.000§	0.250§	0.250§	65.1	1.8
	UO ₂	0.34	-0.052	0.221	0.088	44.7	1.8
	UO ₂	0.22	-0.016	0.176	0.149	42.5	1.8
	UO ₂	0.22	-0.112	0.187	0.063	41.7	1.8
	Cd	-0.11	0.000§	0.233§	0.267§	65.2	1.8
	Cd	-0.22	-0.156	0.177	0.194	56.2	1.8

Mean figures of merit: centric $\langle m \rangle = 0.86$
acentric $\langle m \rangle = 0.78$
overall $\langle m \rangle = 0.82$

*Relative occupancies, at an arbitrary overall scale factor.

[†]Distance from centre of molecule.

[‡]Normal crystallographic residual, $R_c = \frac{\sum (|F_{PH} - F_P| \cdot |F_H|)}{\sum |F_{PH} - F_P|}$.

§Parameter not refined.

¶Parameters set equal.

at the threefold axis (radius 57.4 Å) and the other at the inner end of one of the long helices at radius 43.3 Å. We infer the presence of two cysteines both accessible to solvent, though not in exposed positions.

Second, we may learn more about the metal-ion binding properties of apoferritin, with a view to understanding the catalytic action in incorporating iron into its crystalline micelle^{17,18}. The uranyl nitrate derivative we chose specifically to look for carboxyl (or other) groups which might bind iron and help nucleate the micelle structure. We find that both cadmiums are displaced by uranyl, although we cannot see a uranyl peak near the threefold axis. In addition, there are three uranyl groups on the inner surface of the protein shell, at radii 41–45 Å. These potential iron-binding sites do not seem close enough to be able to cooperate in nucleation or catalysis of oxidation. The quality of the 6-Å map, we believe, augurs well both for the protein structure determination at higher resolution, and for the elucidation of the catalytic role of apoferritin.

We thank the Science Research Council, the Medical Research Council and the Royal Society for support, and Mrs J. Brentnall for technical assistance. We thank Sheffield University and the Manchester Regional Computing Centre for the use of computing facilities.

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Received March 12; accepted April 21, 1975.

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matters arising

Chandler wobble and viscosity in the Earth's core

VERHOOGEN¹ has recently revived viscous dissipation at the core-mantle boundary as a possible energy sink for the Chandler wobble. His result seems to be based on a misunderstanding of the work of Jeffreys² (page 257) and Munk and MacDonald³ (page 170). He correctly points out that Jeffreys' equation (13) requires multiplication by a factor $4\pi/\omega$, where ω is the diurnal frequency. In fact it contains a further error, in that $(l_1^2 + m_1^2)$ should be replaced by $(l_1^2 + m_1^2)/\omega^2$. This is true in the fifth (1970) edition of *The Earth*, as well as in the fourth, cited by Verhoogen. Contrary to Verhoogen's statement, however, this error is not carried into the numerical calculation leading to Jeffreys' equation (14).

In agreement with other authors, Verhoogen takes the viscous dissipation per unit area at the core-mantle interface to be of the order $\rho V^2(v\omega)^{1/2}$, where ρ is the density and v is the kinematic viscosity of the core just below that boundary, and V is the velocity of the core relative to the mantle in the Chandler wobble mode. Let γ be the angle between the instantaneous axis of rotation of the mantle and the angular velocity (more properly, the total vorticity vector) of the liquid core, and let α be the amplitude of wobble of the mantle. Then $V = \gamma a \omega$, where a is the radius of the core-mantle boundary. In Jeffreys' notation $\gamma = (l_1^2 + m_1^2)^{1/2}$ and $\alpha = (l^2 + m^2)^{1/2}$. Verhoogen errs in assuming that γ can be identified with α .

In fact, as Jeffreys and Vicente⁴ showed, the liquid core hardly participates at all in wobble in the Chandler mode, that is, its vorticity vector remains nearly aligned with $\mathbf{H}$, the invariable total angular momentum of the wobbling Earth. Clearly γ is the amplitude of the nutation in space accompanying the Chandler wobble. If, as Verhoogen suggests, γ is of the same order as α ($\approx 0''.14$), this nutation could hardly have escaped detection by astronomers! Rochester *et al.*⁵, in their equations (10) and (11), show explicitly that

$$\gamma = |\boldsymbol{\omega} \times \mathbf{H}| / |\mathbf{H}| \simeq \alpha \sigma / \omega$$

where $\sigma = \omega/435$ is the frequency of the Chandler wobble. (This relationship between γ and α was implicitly taken into account by Jeffreys in the fourth and fifth editions of *The Earth*, to correct his equations (13) and (14) as they were

given in the third (1952) edition.) It follows that $V = \alpha a \sigma$, in agreement with Munk and MacDonald. Then the rate of dissipation of wobble energy by viscous core-mantle coupling is

$$F = 4\pi \rho a^4 \sigma^2 \alpha^2 (v\omega)^{1/2}$$

This differs by a factor $(\sigma/\omega)^2$ from Verhoogen's equation (1), and leads to Jeffreys' equation (14) when the appropriate numerical values are substituted.

Verhoogen's estimate of the viscosity required to damp the Chandler wobble is therefore too small by a factor $(\omega/\sigma)^4$, that is, viscosities of the order of those calculated by Gans⁶ and Leppaluoto⁷ ($\sim 10^{-5}$ to 10^{-6} m² s⁻¹) fall short of what is needed to damp the wobble by a factor of order 10^{10} . The situation is still as it was left by Jeffreys and by Munk and MacDonald, and we must look elsewhere for the energy sink for the Chandler wobble.

I am grateful to Lady B. Jeffreys for helpful comments on the notation adopted in *The Earth*.

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¹ Verhoogen, J., *Nature*, **249**, 334-335 (1974).

² Jeffreys, H., *The Earth*, fourth ed. (Cambridge University Press, Cambridge, 1959).

³ Munk, W. H., and MacDonald, G. J. F., *The Rotation of the Earth* (Cambridge University Press, Cambridge, 1960).

⁴ Jeffreys, H., and Vicente, R. O., *Mon. Not. R. astr. Soc.*, **117**, 142-161 (1957).

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⁶ Gans, R. F., *J. geophys. Res.*, **77**, 360-366 (1972).

⁷ Leppaluoto, D., thesis, Univ. California (1972).

VERHOOGEN¹ has attempted to revive the idea that the viscosity of the core can account for the observed damping of the Chandler wobble. There is, however, an error of principle in Verhoogen's estimation of relaxation time, τ (his equation (3)): the angle α between the axes of rotation of core and mantle has been erroneously assumed to be equal to the angular amplitude, α_w , of the Chandler wobble.

As the core remains approximately motionless, that is, it does not participate in the Chandler wobble², the direction of the rotation axis of the core almost coincides with that of the Earth's total angular momentum vector, $\mathbf{H}$. So α is nearly equal to the angle between the rotation axis of the mantle and $\mathbf{H}$ (the 'sway' amplitude according to Munk and MacDonald's³ terminology), which is known to be $[\sigma/(\sigma - \omega)]\alpha_w \approx (\sigma/\omega)\alpha_w$. Here, ω and σ are the diurnal and Chand-

ler frequencies, respectively. If a is the core radius, the differential velocity between the core and mantle is therefore given by $V = \alpha a \omega = \alpha_w a \sigma$, which is just in agreement with Munk and MacDonald's formula. The wobble energy, E_w , of the mantle is given by

$$E_w = (1/2)A_m[(C_m - A_m)/C_m]\omega^2\alpha_w^2$$

Thus, Verhoogen's expression for τ turns out to be in error by a factor of $(\omega/\sigma)^2 \approx (430)^2$.

Using the most recent and accurate determination of polar motion⁴, one of us (Y.S.Y.) has found that the value of the damping factor, Q , lies between 40 and 60. The corresponding limits on τ are 15 and 23 yr. Assuming that $\tau = 20$ yr, we obtain an estimated value for the kinematic viscosity of the core of about 5×10^8 cm² s⁻¹. Such a value is regarded by most geophysicists as extremely high⁵. It has to be concluded, therefore, that damping of the Chandler wobble is unlikely to be attributable to core viscosity.

Note added in proof: Verhoogen's and our considerations do not depend on the excitation mechanism of the Chandler wobble and have only a kinematic character. One can consider the value $\alpha_w a$ as the amplitude of the displacement of the mantle relative to the core in the course of the Chandler period σ^{-1} . For this reason the differential velocity

$$V \sim \alpha_w a \sigma$$

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³ Munk, W. H., and MacDonald, G. J. F., *The Rotation of the Earth* (Cambridge University Press, Cambridge, 1960).

⁴ Fedorov, E. P., *et al.*, *Motion of the Earth's Pole from 1890 to 1969* (Naukova Dumka, Kiev, 1972).

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VERHOOGEN REPLIES—Viscous damping of the Chandler wobble depends on two angles. The first, α_w , is the angle between $\mathbf{e}_3$ (a unit vector along the mantle's principal axis of inertia) and $\boldsymbol{\omega}_m$, the angular velocity of the mantle. The second angle is the angle α_F between $\boldsymbol{\omega}_m$ and $\boldsymbol{\omega}_c$, the

vorticity (assumed to be uniform) of the core. When α_w and α_F are of the same order, viscous damping of the wobble is possible, as shown. It is generally argued that α_F is equal to the angle between ω_m and L_m (L is angular momentum) which is of the order of $\alpha_w/400$. This would indeed be the case if the Chandler wobble were excited by earthquake-related displacements that shift the axis of the figure of the mantle by an angle α_w while leaving ω_c , L_m , and L_c undisturbed and aligned along L , the total angular momentum of the Earth.

But do we know what ω_c is doing? Has it been completely ruled out that the Chandler wobble may be excited¹ by electromagnetic impulsive torques? Such torques would shift ω_m , ω_c , L_m , and L_c , with only the sum $L_m + L_c$ remaining invariant; the angle α_F between ω_m and ω_c could be much larger than the angle between ω_m and L_m . Alternatively, if earthquake-related displacements at the core-mantle boundary shift the axis of figure of the core cavity, wouldn't inertial coupling make ω_c tend towards the new container axis, thus departing from the invariant direction of L ?

At present it is not known what excites the Chandler wobble nor what dampens it, so the assertion that α_F is necessarily much smaller than α_w seems unwarranted; thus viscous damping remains possible.

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Enhanced translational motion of *Leptospira* in viscous environments

Cox and Twigg¹ theorised that the spinning motion frequently observed during non-translatory movement of *Leptospira icterohemorrhagiae* (strain 3H) is an illusion created by a rapid circular motion of the two ends of the organism while the body itself remains stationary.

Our studies also indicate that the rotation reported for free-floating *L. interrogans (biflexa)* B-16 is indeed an optical illusion arising from a rapid bipolar 'wobbling' movement. Thus, we confirm their results and extend observations on this interesting form of unicellular movement. Note, however, that this non-translatory motion predominates, and is often described in textbooks as 'typical' for leptospires observed in laboratory-produced culture media. One might conclude that movement for these organisms serves little purpose save stirring up the immediate surrounding environment, but advantages gained would seem hardly to balance the energy expenditures

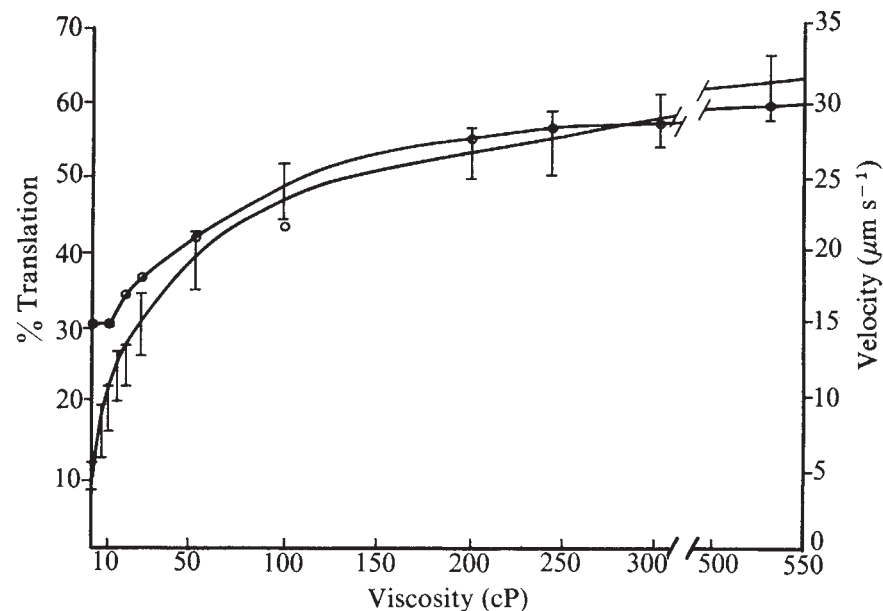


Fig. 1 Left ordinate: percentage of leptospires showing translational movement in SM-4 medium supplemented with methyl cellulose (I). Leptospiral culture (300 μ l) was mixed with 1.0 ml viscosity test solution. Two hundred motile organisms were counted per observation and eight observations made for each viscosity indicated. The range of the eight observations is indicated on the graph. Right ordinate: average velocity in SM-4 medium supplemented with methyl cellulose (O). Velocity was determined by the method of Schneider and Doetsch². Twenty velocity determinations were made at each viscosity indicated, with the ten greatest velocities used to determine average velocity.

likely to be required for such a motion.

As Schneider and Doetsch² reported that many kinds of flagellated bacteria increase their translational velocity ($\mu\text{m s}^{-1}$) in a more viscous milieu, we determined whether more efficient leptospiral movement would result from an increase in the viscosity of the environment.

Translational movement was notably enhanced in viscous solutions. In SM-4 medium supplemented with methyl cellulose, there was a marked increase both in the number of individual organisms exhibiting translational movement, and in the velocity they attained (Fig. 1). Maximum percentage translation and velocity were not achieved until viscosity exceeded 300 centipoise (cP). Leptospires suspended in semi-solid Ionagar (0.35–0.50%, w/v) behaved similarly. The enhanced translatory movement observed in such highly viscous solutions contrasts markedly with flagellated bacteria which show an increase in velocity as viscosity is elevated to around 2–5 cP (depending on the organism and its flagellar arrangement), but then exhibit a rapid decrease in velocity beyond this range². This difference in translational ability in highly viscous solutions is doubtless related to the fact that eubacteria propel themselves by means of exoflagella, whereas leptospires possess endoflagella which are, most probably, their organelles of locomotion³.

Jahn and Landman⁴ stated that hydrodynamic efficiency of spirochetes can be

calculated from the ratio of the area pushing forward (end cross-sectional area) to the total area pushing forward and backward (the area of the front end plus the area of the posterior exposures of the coils).

The overall structure of leptospires is amenable to a viscous environment, namely, a minimum area pushing forward resulting from a small cross-sectional area, a maximum area pushing backward as a consequence of their spiral shape, and the ability to produce travelling waves as a result of their flexibility.

Extremely viscous environments would, on the other hand, have an antagonistic effect on flagellated bacteria since they have a greater diameter than leptospires, and because increased viscosity would have a damping action on their flagella.

It is evident that *Leptospira* exhibits more efficient translational movement in viscous or semi-solid media than in normal laboratory formulations. It is suggested that if efficiency of translational motion confers a selective advantage to leptospires, then their behaviour in semi-solid or viscous environments may typify natural movements rather than those observed in ordinary laboratory cultures.

These observations are important in that they provide insights into how pathogenic leptospires may negotiate the viscous fluids, mucosal surfaces, and intracellular spaces in the host's body, and conceivably into how free-living ones solve problems such as traversing

the bacterial and algal slimes and mire of their environment.

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Function of cochlear hair cells

RYAN and Dallos¹ reported the effect of the ototoxic drug kanamycin on behavioural audiograms of chinchillas. In most cases, histological examination with the light microscope showed that 100% of outer hair cells (OHCs) were missing from much of the basal turn of the cochlea, whereas the row of inner hair cells (IHCs) seemed to be intact. They found quite a good rank order correlation between the extent of OHC damage and the frequency at which the drug-induced threshold shift begins. In two of the cases they illustrated, however, major discrepancies are obvious. In one case, the threshold shift begins at 3–4 kHz yet 100% of OHCs are present up to the 7 kHz region. Their whole thesis of facilitation of IHC function by the OHCs depends on the stated assumption that IHCs remaining in the basal turn, which appear normal under the light microscope, are functionally normal. Yet in this case, there are OHCs scored as intact but in the 4 kHz or 7 kHz region are obviously functionally abnormal. It seems unjustified to assume that IHCs in these regions are similarly not functionally impaired, and to minimise the importance of cytoplasmic changes visible only under the electron microscope. As Wersäll² has noted "... it is remarkable how well preserved the cuticle and the hairs might be in a cell with advanced cellular protoplasmic degeneration."

Furthermore, although I agree with Ryan and Dallos that species differences may be important, it is pertinent to discuss the study of Kiang *et al.*³ in this regard. Using the same drug, they produced almost identical lesions in the cat—massive to complete loss of OHCs and 'intact' IHCs. Yet, in recording VIIIth nerve unit activity, they were unable to detect any units which would respond to frequencies found in these regions of the cochlea. Instead, non-spontaneous, non-responding units were detected (using electrical stimuli) in the high frequency part of the nerve. Here, where there were 'intact' IHCs only, the

thresholds were increased beyond the range of their equipment (80–100 dB shift). Obviously, these IHCs were functionally abnormal. It is clear that, in contrast to Kiang's cats, Ryan and Dallos' chinchillas could actually hear these frequencies. It seems unreasonable, however, to assume that this detection was mediated by functionally normal IHCs. The hypotheses of IHC and OHC function based on their arguments, although attractive, are correspondingly weakened in the absence of sufficient proof of the viability of the IHCs in their kanamycin-treated animals.

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² Wersäll, J., in *Basic Mechanisms in Hearing* (edit. by Møller, A. R.), 235 (Academic, New York, 1973).

³ Kiang, N. Y.-S., Moxon, E. C., and Levine, R. A., in *Sensorineural Hearing Loss* (edit. by Wolstenholme, G. E. W., and Knight, J.), 241 (Churchill, London, 1970).

RYAN AND DALLOS REPLY—Manley¹ presents a criticism of our suggestion² that outer hair cells (OHCs) may facilitate inner hair cell (IHC) function at low sound levels on the basis that our assumption pertaining to the normality of those IHCs remaining in our preparations is incorrect. His arguments are based on two issues one of which, we feel, is inadmissible, the other irrelevant.

First, he cites the less than perfect correlation between extent of OHC lesion and cutoff frequency of hearing loss as a critical weakness. In fact, such correlations are by nature less than exact, because one can only rely on average relationships between cochlear location and corresponding frequency (such frequency maps are not even available for chinchillas). Because of the variability of the cochlear length (about 2 mm variability in chinchillas) and as hair cell counts progress from apex to base, it is hazardous to assume, as Manley does, that in an individual animal one can uniquely determine the exact location of a given, relatively high, frequency. In other words, Manley's objection would have merit only if an accurate frequency map were available for each animal, and if on that basis discrepancies were still found to exist.

The second objection, namely that in the single unit work of Kiang *et al.*³ on the cat, damage patterns 'similar' to ours resulted in greater losses—thus our animals could not have had functioning IHCs—simply lacks a logical basis. Aside from possible species differences that make comparisons tenuous, one must ask the question: What is the best

means of establishing that a preparation functions? Clearly, to assess the degree of damage to the hearing organ, one may be on safer grounds by asking what the animal can hear, not by merely studying morphology or the responses of single neurones. Accordingly, in our animals the removal of OHCs resulted in a threshold shift of between 40 and 50 dB. This shift was maintained in magnitude but extended over differing frequency bands which were correlated with the configuration of OHC loss. One would think that variable damage to the IHCs would have resulted in different degrees of loss not merely the same loss covering different extents of the high frequency range.

Finally, Manley's quote from Wersäll's² paper is out of context. Wersäll emphasised the poor correlation between signs of damage seen in light and electron microscopy, when such comparisons are made soon after the antibiotic insult to the cochlea. In fact, there is reason to believe that originally damaged cells disappear in time, and that after long survival (as is the case in our study) the surface preparation technique would give a fair indication of the state of the hair cells.

Evanston, Illinois 60201

¹ Manley, G. A., *Nature*, **255**, 657 (1975).

² Ryan, A., and Dallos, P., *Nature*, **253**, 44–46 (1975).

³ Kiang, N. Y.-S., Moxon, E. C., and Levine, R. A., in *Sensorineural Hearing Loss* (edit. by Wolstenholme, G. E. W., and Knight, J.), 241 (Churchill, London, 1970).

⁴ Wersäll, J., in *Basic Mechanisms in Hearing* (edit. by Møller, A. R.), 235 (Academic, New York, 1973).

θ -Positive cells in nude mice born from homozygous *nu/nu* mother

Two types of θ^+ lymphocytes have been found in the lymphoid tissues of athymic nude mice. Some have a density of θ similar to that of peripheral T lymphocytes of normal mice and make up about 1% of nude mouse spleen cells^{1,2}. Others have a low density of θ and make up about 20% of nude mouse spleen cells^{2,3}.

Nude mice studied so far were from heterozygous *nu/+* parents and as has been pointed out^{3,4} there may be an influence of maternal and normal littermate T cells or thymic humoral factors on the cells of the nude littermate.

Litters from homozygous *nu/nu* mothers usually do not survive. We recently had the opportunity, however, to study three nude mice born from a homozygous *nu/nu* mother and an heterozygous father and nursed by the mother. We found that their content of both strong and weak θ -positive lymphocytes was comparable with that of their mother and *nu/nu* mice from heterozygous *nu/+* mothers^{2,3}. The results are not completely unequivocal as one

normal littermate was born as well. But, the main source of mature T cells or thymic factor during embryonic life should be the mother¹. Thus these findings support the concept that the weak θ -positive lymphocytes may be prethymic T-cell precursors^{2,3} as the normal thymus or its products are not essential for their appearance in nude mice. They also suggest that nude mice may not be totally devoid of T-cell function.

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- ¹ Raff, M. C., *Nature*, **246**, 350 (1973).
² Loor, F., and Roelants, G. E., *Nature*, **251**, 229 (1974).
³ Roelants, G. E., et al., *Eur. J. Immunol.*, **5**, 127 (1975).
⁴ Raff, M. C., *Nature*, **251**, 184 (1974).
⁵ Humber, D. P., Pinder, M., and Hetherington, C. M., *Transplantation*, **19**, 91 (1975).

Y chromosome and aggression in mice

GENETIC differences in the aggression of mice have been attributed to the Y chromosome¹. As such a claim has obvious implications in the current controversy over the behavioural correlates of the XYY karyotype in man, alternative explanations must be considered.

The evidence depends on reciprocal differences in F_1 hybrids, where male hybrids scored closer to the male than the female parent. Although this rules out X chromosome inheritance, it does not rule out maternal effects. The authors state that "Because the most aggressive hybrids developed in the uterine and postnatal environment of the less aggressive C57BL/10/Bg strain, these maternal conditions seem to make no major contribution to the aggressive behaviour of these strains and hybrids". This assumes that maternal effects always make the offspring more like the mother, that is, that any maternal and additive genetic effects are always in the same direction. But there are no *a priori* reasons why this should be so, especially when one is considering a trait such as aggression, that they measured only in males. Clearly, if maternal effects are acting in an opposite direction to additive genetic effects, we have a situation of

apparent paternal inheritance and Y chromosome effects.

Data do exist² that demonstrate such maternal effects on the steroid responses of C57BL/10J and DBA/2 mouse strains, which presumably are fairly closely related to the C57BL/10/Bg and DBA/1/Bg used here. In the non-handled females there was a large positive additive genetic effect, where the DBA genotype acted to raise the corticosteroid response, and an equally significant negative maternal effect, where the DBA maternal environment lowered the response. Such a complex form of interaction is not merely a statistical artefact, but has definite evolutionary advantages in situations where natural selection favours an intermediate level of performance.

Fulker³ gives further examples of such "maternal buffering" and in Table 1 I have reanalysed the data under discussion by his method to give the four parameters, m = mean $[d]$ = additive genetic effect, $[d_m]$ = maternal effect and $[h]$ = dominance.

Using *t*-tests, m and $[d]$ turn out to be highly significant ($P < 0.001$) in both experiments, the large $[d]$ values contradicting the comment of Selmanoff, Jumonville, Maxson and Ginsburg¹ that no autosomal contribution was found for aggression. Admittedly the present model does separate additive and maternal effects more clearly than their scaling approach. $[d_m]$ is more significant ($P = 0.02$) in the larger experiment, though it should be noted that in both experiments it is only one-third the size of $[d]$, the additive genetic component. $[h]$ is of borderline significance in both experiments, though its fluctuations in sign between experiments are puzzling and should have been mentioned.

Although such maternal and genetic influences in opposite directions could explain the data and, in view of the previous evidence of others^{2,3} are possibly more likely than Y chromosome inheritance, the latter cannot yet be ruled out completely with these data, as $[d_m]$ and $[d_Y]$ (for the effect of a Y chromosome) have identical coefficients^{4,5} in these four generations and cannot be separated. Further experiments are the only way of deciding the issue. No examples exist in mice, but, working on *Drosophila* behaviour, I have described an extension of the m , $[d]$, $[d_m]$ and $[h]$ model to the situation where F_2 s and backcrosses are tested⁵. I have discussed several examples

of complex interactions between maternal and autosomal effects that could be detected unequivocally with these extra generations. Until such further generations are tested in these mice, the hypothesis of Y-linked inheritance of aggression in mice remains unproven.

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- ¹ Selmanoff, M. K., Jumonville, J. E., Maxson, S. C., and Ginsburg, B. E., *Nature*, **253**, 529 (1975).
² Treiman, D. M., Fulker, D. W., and Levine, S., *Dev. Psychobiol.*, **3**, 131 (1970).
³ Fulker, D. W., *Behav. Genet.*, **1**, 119 (1970).
⁴ Mather, K., and Jinks, J. L., *Biometrical Genetics* (second ed.) (Chapman and Hall, London, 1971).
⁵ Hay, D. A., *Heredity*, **28**, 311 (1972).

SELMANOFF *et al.* REPLY—HAY¹ suggests that there are autosomal effects, that a reversal of dominance occurs, and that our differences in reciprocal F_1 s are attributable to factors other than the source of the Y chromosome.

We took these possibilities into account, and suggested that autosomal factors and maternal factors may be involved (see our fifth paragraph, ref. 2). Similarly, what Hay interprets as reversals in dominance are described in our fourth paragraph, and explanations for these changes are proposed.

Hay's explanation of the reciprocal differences in aggression of F_1 hybrids depends on the assumptions made in applying the diallel model³ and on Fulker's⁴ findings of genetic and maternal influences acting in opposite directions. In using a Mendelian analysis, we take a more parsimonious approach to the assumptions that must be made. There are, in addition, very few examples where maternal and genetic influences act in opposite directions; whereas, there is now a growing body of evidence for the involvement of the Y chromosome in the inheritance of particular characteristics⁵. Aggressive behaviour is complex and undoubtedly has multiple genetic determination. As mentioned in our paper, there is no evidence for Y chromosome inheritance with respect to differences in aggressive behaviour where the DBA/2/Bg strain is involved⁵. We have, therefore, suggested that the most parsimonious explanation for all of our findings is that there is genetic variation in the Y chromosome of the mouse, such that some Ys, but not others, make substantial contributions to aggressive behaviour, as do autosomal genes and early experience. Further research is needed to substantiate this hypothesis.

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- ¹ Hay, D. A., *Nature*, **255**, 658 (1975).
² Selmanoff, M. K., Jumonville, J. E., Maxson, S. C., and Ginsburg, B. E., *Nature*, **253**, 529 (1975).
³ Mather, K., and Jinks, J. L., *Biometrical Genetics* (second ed.) (Chapman and Hall, London, 1971).
⁴ Fulker, D. W., *Behav. Genet.*, **1**, 119 (1970).
⁵ Selmanoff, M. K., Maxson, S. C., Ginsburg, B. E., *Behav. Genet.* (in the press).

Table 1 Estimates for the four-parameter genetic model of mouse aggression

	Initial experiment	d.f.	Replication	d.f.
m	19.65 ± 2.76*	36	21.35 ± 2.57	123
$[d]$	20.90 ± 4.17	36	18.60 ± 3.17	123
$[d_m]$	-6.25 ± 3.13	18	-4.55 ± 1.86	83
$[h]$	8.10 ± 4.17	36	-6.10 ± 3.17	123

* ± s.e.

Details of these parameters and how to calculate them and their s.e. are given by Fulker³ (except that his formulae for s.e. of $[d_m]$ and s.e. of $[d]$ on page 121 are accidentally transposed) and more fully by Mather and Jinks⁴.

reviews

THE Tercentenary of the founding of Britain's oldest scientific institution, the Royal Greenwich Observatory (RGO), provides the occasion for commemoration and celebration, and a most appropriate opportunity for some more permanent record of its achievements. There is to be a full programme of commemorative and celebratory functions: special services, lectures, visits, exhibitions and publications—including Professor McCrea's admirable, but short, historical survey of the RGO. The RGO itself was moved to Herstmonceux Castle in Sussex in the 1950s, when the old Royal Observatory was transferred to the care of the National Maritime Museum as the basis for a museum of astronomy and navigation. The buildings, including Flamsteed House as designed by Wren in 1675, have been magnificently restored, with almost all of the old instruments displayed *in situ*; the whole forms a worthy monument to 300 years of scientific endeavour and accomplishment. This three-volume history* must be regarded as contributing towards the written record that will complement the material record. But the three volumes differ so markedly in approach, style and content that they could almost have been prepared independently; and, certainly, each could stand on its own. None of the three authors was ever a member of the observatory staff, though Eric Forbes was one of the earliest vacation students at Herstmonceux.

Since he left the field of astronomy for that of the history of science,

**Greenwich Observatory: The Royal Observatory at Greenwich and Herstmonceux, 1675–1975*. Vol. 1: *Origins and Early History (1675–1835)*. By Eric G. Forbes. Pp. xv+204. Vol. 2: *Recent History (1836–1975)*. By A. J. Meadows. Pp. xi+135. Vol. 3: *The Buildings and Instruments*. By Derek Howse. Pp. xix+178. (Taylor and Francis: London, April 1975.) £25.00 the set.

Forbes has made for himself an enviable reputation for scholarship coupled with the ability to collate, interpret and present the immense amount of material he so assiduously assembles; in particular, he has studied the lives and works of Flamsteed and Tobias Mayer in depth. As may thus be expected, Volume 1 is a scholarly, authoritative and fully documented account not only of the history of the Greenwich Observatory to 1835 (the year in which Pond retired as Astronomer Royal to be suc-

tory was founded. He takes care to give, for all the major contributors, sufficient technical information for their proper appreciation. But he does more: he seeks to portray the motives, scientific or otherwise, and the personalities of those concerned, as for instance in the famous, or infamous, quarrels between Flamsteed and his contemporaries Newton and Halley. There is so much material available, in the archives of the RGO and elsewhere, that selection and interpretation present

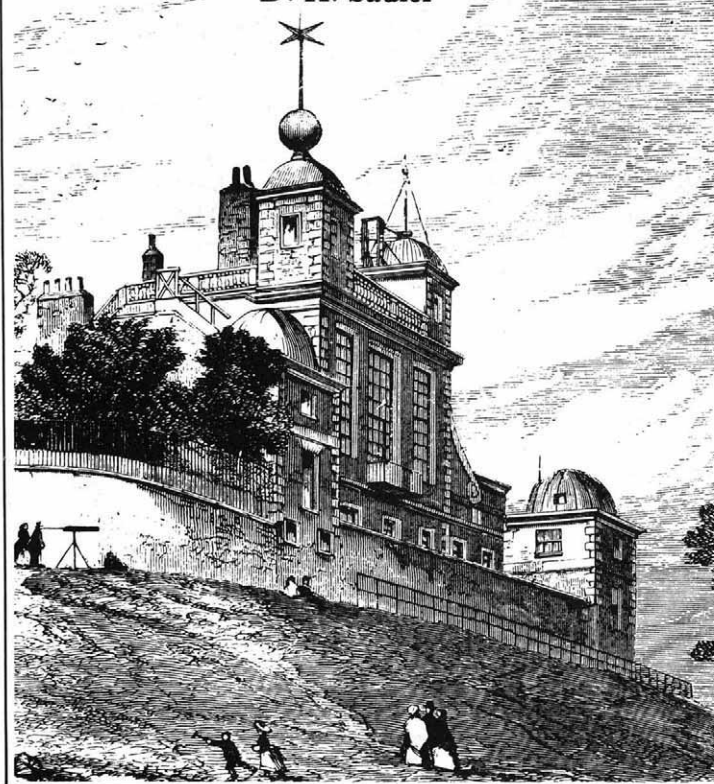
considerable difficulty. Forbes has succeeded in compressing the whole material into a reasonable length without significant omissions; and his fluent style makes the sometimes heavy content easy to read. Uncharacteristically, there are a few undetected errors in some of the technical descriptions, but they do not detract from a most valuable contribution to historical research.

In Volume 2, Jack Meadows, whose book *Science and Controversy* is well known, adopts quite a different approach in that he analyses the growth of the observatory since 1835 as an institution rather than as a study of the activities of the Astronomers Royal. His treatment is more general, somewhat less technical, and consequently rather lighter; he writes fluently, interestingly and readably. But he gives the impression that he has not always consulted the authoritative sources and that some of his comments are

superficial. The main work of the observatory is covered in three chapters concerned with developments in positional astronomy, longitude and time, and astrophysics and geophysics. These are straightforward, factual, accounts which are fully adequate for general reading. During most of the 140 years covered by this volume the emphasis was firmly on positional astronomy, to which Greenwich made a not inconsiderable contribution; more reference might well have been made to this, with mention of the end-product of published observations and star cata-

Complementary material

D. H. Sadler



ceeded by Airy) but also of the contemporary intellectual scene and astronomical background. The somewhat colourful events leading to the provision of the observatory are followed by detailed accounts of its main work and achievements under the direction of the six Astronomers Royal from Flamsteed to Pond; he gives full coverage to the story of Maskelyne's introduction, in 1766 for the year 1767, of *The Nautical Almanac*—thus providing the practical, astronomical, solution of the problem of determining longitude at sea, for which purpose the observa-

logues. Some readers may also be misled by the curious confusion between UT2 and Ephemeris Time, which is erroneously stated to be dependent on the rotation of the Earth. They will, however, find the first and last chapters of more interest; these deal, respectively, with the Astronomers Royal and their staffs (what a wealth of material from which to choose) and with the changing status of the observatory. Perceptively written, they go beyond the mere recording of events, and analyse changes and trends and their future significance. The last 10, 20 and 30 years have seen fundamental changes—in control, direction, function, equipment and staffing—greater than in the previous 270 years; and it is perhaps premature to do more than record them. The author's assessments and views as to the future will be studied with interest, even if not with universal agreement.

In Volume 3, Derek Howse has had the more straightforward—but not less onerous—task of describing the buildings and instruments at Greenwich. He has produced a definitive record that is worthy, in every respect, of the traditions of the observatory. Authoritative, well documented, comprehensive and detailed, it is also admirably planned and presented. The first chapter is devoted to a narrative account of the growth of the buildings, and is supplemented by an appendix showing ground plans at six dates. This is followed by

detailed descriptions of the instruments, arranged in chapters according to design or function; for each is given precise factual data, method of use, and history. Almost every instrument is included in the 130 illustrations, which also include specimens of the published results obtained with the more important of them. The style is direct and economical—and very readable. Altogether, it makes a superb publication which represents many years of patient research and devoted labour by the man mainly responsible for transforming the war damaged and neglected buildings, and their contents, into their present excellent condition. It is appropriate that the Director of the RGO, Dr A.

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Hunter, should have contributed an introduction to this volume. He refers to the pride which members of the Greenwich staffs had in their historic establishment; they had also, and still have, affection. This volume in particular will give them—and many others—universal pleasure. □

High Energy Astrophysics and its Relation to Elementary Particle Physics. Edited by Kenneth Brecher and Giancarlo Setti. Pp. 591. (MIT Press: Cambridge, Massachusetts and London, 1974.) n.p.

THIS book is a collection of the lectures given at the International School of Astrophysics held in Erice, Sicily, in the Summer of 1972. The lectures have taken more than two years to be published although the publishers had hoped to reduce the publication time by printing the book in typescript format. This delay in publication is a major drawback to the book, especially as far as the lecture course on X-ray astronomy by Giacconi is concerned. That subject is not static and three years ago the physics of accretion discs was not fully appreciated. My major criticism of the book, however, is that most of the lectures present personal views of high energy astrophysics and related topics. For example, Hagedorn writes on his theory of strong interactions, implying an upper bound to the temperature during the early stages of the universe; that must be compared with Hoyle's Machian type cosmology

in which he hopes to determine the masses of fundamental particles.

The lectures on observational astrophysics (Arp, Burbidge and Sargent) are, with the exception of that from the first of these authors, less one-sided. Sargent presents a good review of the standard determination of the Hubble constant and the deceleration parameter. Unfortunately, there are many diagrams rather than photographs of galaxies and clusters—it would be nice to see what some of the objects really look like. Arp presents very much his own interpretation of the redshift problem (the possible physical association of objects with high and low redshift) and there are books available which present both sides of this argument.

The school was, and presumably the book is, intended for graduate students in astronomy, but I would hesitate to recommend this volume to that particular group. Some of the contributions (for instance, those by Sargent and Hagedorn) give an extensive list of references, whereas others give no references at all. But as the book is generally very readable it is at least good bedside reading.

Andrew Bolton

Guide to positional astronomy

Positional Astronomy. By D. McNally. Pp. xiii+375. (Muller: London, March 1975.) £8.50, cloth; £4.25, paper.

THERE are some who may feel that a new introductory text on positional astronomy is superfluous, in view of the comprehensive nature of W. M. Smart's *Textbook on Spherical Astronomy*; they would, however, have missed the point of McNally's book. The ground covered (necessarily very similar to that surveyed by Smart) begins with a chapter establishing a background of spherical trigonometry. This is followed by a very clear introduction to coordinate systems, a discussion on methods of time measurement, and a chapter outlining the derivation of first-order corrections for those factors (from refraction to proper motion) which displace the image of a star from its geometrical position. Eclipses, occultations and transits are dealt with, and there is a useful section on plate measurements by the method of dependences. Two chapters cover elementary orbit theory and the determination of the elements of a planetary orbit, and the book concludes with a consideration of the information obtainable from observations of binary star orbits.

Intended for the first year undergraduate as an introduction to the concepts underlying astrometry, the strength of this book lies in the organisation of its material. Each chapter follows logically, introducing further refinements to measurements and techniques as necessary. And the appendices, which summarise many formulae and the relationships between them, are particularly handy for quick reference.

The subject matter is also refreshingly up-to-date, particularly in the chapter on 'time', but it is regrettable that the presentation is not more modern. That shortcoming, and the rather dry style of writing, combine to render *Positional Astronomy* a textbook in the traditional mould, and one which is not easy to read through from cover to cover. With the widely held view among many undergraduates that the study of astrometry is a necessary evil, perhaps it would have been more helpful if the author had taken a more 'popular' approach.

As a text, however, this book succeeds, and it should prove a useful guide to the working astronomer who needs to refer to astrometrical techniques as a means to an end. It may well enjoy more acclaim in this market than in that for which it was originally intended.

Heather A. Couper

Waves and Satellites in the Near-Earth Plasma. (Studies in Soviet Science.) By Ya L. Al'pert. Translated from Russian by Julian B. Barbour. Pp. ix+196. (Consultants Bureau: New York and London, 1974.) \$42.00.

This book reviews two independent phenomena observed in the magnetosphere and the solar wind: first, the way in which the plasma is disturbed when a satellite passes through it, and, second, the nature of the naturally occurring waves.

The first of three chapters outlines what is known about waves in cold and in hot magnetoplasmas: it also reviews the phenomena of particle collection, reflection, and emission from the surface of a satellite and the potential that the satellite finally attains. The second chapter reviews the phenomena that can occur in the neighbourhood of a moving satellite which may have a velocity either greater than, or comparable with, the ion thermal velocity, and a size either greater or less than the Debye length. Although there is mention of the few experiments that have been made on the topic, this chapter is largely theoretical. Chapter 3, concerned with naturally occurring waves and oscillations in the plasma, summarises the numerous experimental results that have been obtained, and gives an outline of their theoretical explanations.

This wide range of topics is discussed within the framework of a single comprehensive theory. The reader is supposed to be acquainted, at least in outline, with the details of the theory, since only the results are quoted; anyone who has that acquaintance will appreciate the skill with which the author brings out the interrelationships between the diverse phenomena.

The book has been competently translated from Russian and provides a very readable and compact account of a complicated subject. It is reasonably up-to-date: of the 186 references cited most are from the past ten years, a few from the past five.

J. A. Ratcliffe

Black Holes, Gravitational Waves and Cosmology: An Introduction to Current Research. By M. Rees, R. Ruffini and J. Wheeler. Pp. 331. (Gordon and Breach: London, February 1975.) £12.80. MY initial reaction on seeing this book was that, as presented, it is rather late on the scene. Though there is the making of a very useful introduction to relativistic astrophysics and cosmology here, the volume seems to have been put together out of various bits and pieces rather hurriedly without sufficient attention having been given to making the different pieces blend smoothly, or to bringing them all up to date. But that is not to say that the pieces themselves are inadequate.

Chapters 1-10 (covering chiefly pulsars,

black holes, QSOs and gravitational radiation) provide the largest discrete chunk, and are, according to the Preface "based on" a report by Ruffini and Wheeler which appeared in an ESRO book (SP-52) in 1971, "as updated for the present book". It would need a keen eye to spot much evidence of this updating, however, possibly because it had originally been intended to reproduce this part of the book from the ESRO publication in facsimile form. Several more recent papers are included in facsimile as appendices, but it seems a pity that the publishers have gone to the expense of resetting the first 10 chapters without obtaining substantial updating from the authors. Presumably the latter chapters,

Universal phenomena

covering cosmology, galaxy formation, and so on, represents Rees' contribution. Most of this material has suffered less from publication delays, since there have been no recent dramatic developments in cosmology, to rival the advances made in the investigation of the black hole phenomenon over the past couple of years. The attempt to make up for that deficiency by including facsimiles of a dozen or so papers published in 1970, 1971 and 1972 is clumsy, and will not be a great help to the beginner.

I am sure that a delay in publication of another few months would have been worthwhile and would have allowed time to produce a thoroughly modernised book. The three authors are all closely involved in the work they are describing and even in the present packaging their contributions provide a valuable volume for students of such matters, especially those new to the field. I hope, however, that the publishers will enlist the aid of one of the authors in producing a more homogeneous and up-to-date second edition.

John Gribbin

Cosmic Rays: Variations and Space Exploration. By L. I. Dorman. Pp. xv+675. (North Holland: Amsterdam and Oxford; American Elsevier: New York, 1974.) Dfl.215; \$82.75.

PROFESSOR L. I. Dorman has made a massive contribution to research on cosmic ray time variations and this first volume of a revised and enlarged version of his previous work on the subject provides another scholarly tome. The book deals with the interpretation of experimental results in respect of the terrestrial atmospheric and magnetic correcting factors which must be applied to intensity measurements, and the second volume will emphasise the physics of cosmic ray modulation in interplanetary space.

Most of the energy density in cosmic radiation is carried by particles which undergo a large reduction in their intensity as they penetrate the magnetic field of the solar wind, so an interpretation of the associated time variations is necessary in order to return to the charge composition and energy spectrum of the relativistic nuclei in interstellar space. It is only when this demodulation has been achieved that a proper discussion of the origin of cosmic rays can proceed. Much of the important work determining the near-Earth values of the energy spectra for electrons, protons and heavy nuclei as a function of time, has been performed using satellite and balloon-borne instruments since the preparation of the Russian edition of this volume, although Dorman documents the relevant work up to 1966-67. Determination of the anisotropy, solar cycle variation and Forbush or magnetic storm associated effects can, however, still only be achieved to a high degree of statistical accuracy using ground-based monitors of the secondary radiation. The first harmonic of the daily variation is only about 0.2% in amplitude and the second harmonic is rather less. Yet these harmonics are related to large-scale particle streaming and particle density gradients in interplanetary space and their interpretation yields information on the complete three-dimensional transport of energetic particles throughout the solar cavity.

It is in the description of the apparatus used to make these observations, of the meteorological corrections to the magnitude of the variations and of the use of the geomagnetic field and secondary particle multiplicity to determine the directional and energy dependence of the primary time variations that this book excels. Each chapter describes the historical development of the correction and interpretation techniques. Exhaustive references are given, together with several appendices containing tables of particle cutoff energies for arrival in the geomagnetic field, information on viewing directions of cosmic ray telescopes corrected for geomagnetic bending, and meteorological correction coefficients for changes in atmospheric pressure, temperature and layer height. In the text, details of schemes for calculating the sealevel secondary products of a primary cosmic ray collision in terms of the physics of the π - μ and electromagnetic cascade are given.

Dorman has written an encyclopaedic account of a research method, developed mainly in the period 1950-65, which should become the standard textbook of all who need this particular tool.

J. J. Quenby

obituary

Percy L. Julian, the research chemist and leader in the fight for civil rights, died on April 19 in Illinois at the age of 76.

Dr Julian, the grandson of a slave, was best known for his work in steroid chemistry, including the commercial synthesis of intermediates for use in the large-scale production of cortisone drugs. His work on soyabean led to the isolation of a soya protein that became the basis of Aero-foam, the extinguisher which saw action in World War II. His research also resulted in the quantity production of soya sterols, for use as steroid intermediates. He gained wide notice for his synthesis of physostigmine, a drug used to treat glaucoma.

Julian was engaged in extensive work for the National Association for the Advancement of Coloured People. Among many awards were the Spingarn Award from NAACP in 1947, election to the National Academy of Sciences in 1973, and the Proctor Prize from Sigma Xi, the science research society, in 1974. At the time of his death, he was director of the Julian Research Institute in Illinois.

Stuart Mudd, a microbiologist who retired in 1959 as professor emeritus of microbiology at the School of Medicine in the University of Pennsylvania, died on May 6 at the age of 81.

Mudd had been a faculty member at Pennsylvania since 1929, after having graduated from Princeton in 1916, and gaining an MA at Washington University (St Louis) in 1918 and an MD from Harvard Medical School in 1920. At Pennsylvania, he founded and served as chairman of the department of microbiology in the School of Medicine. He served as president of the International Association of Microbiological Societies from 1958–62 and was a founder member and vice-president of the World Academy of Arts and Science. During World War II, he served on a technique for freeze-drying blood plasma, which proved to be of considerable value in caring for the wounded.

announcements

Appointment

C. G. Phillips, FRS, has been appointed to the chair of anatomy in the University of Oxford.

International meetings

July 6–13, **Sedimentology**, Nice (Professor J.-P.H. Martin, Sedim Nice 75, 48 avenue Jean Lorrain, 06300 Nice, France).

July 28–30, **Conduction and breakdown of dielectric liquids**, Delft (Dr J. M. Goldschvartz, Chairman, Organising Committee, Department of Applied Physics, Delft University of Technology, Lorentzweg No. 1, Delft, The Netherlands).

Miscellaneous

Student competition. *Preventive Medicine*, the official Journal of the American Health Foundation, has announced a student competition for the best paper (which will be published) on preventive medicine. All students (except postdoctoral) currently enrolled in schools of medicine, dentistry, public health, pharmacy, podiatry or osteopathy are eligible. Prizes of \$300 and \$100 (runner-up). Deadline December 1. Further information: *Preventive Medicine*, Editorial Office, American Health Foundation, 1370 Avenue of the Americas, New York, New York 10019.

Person to Person

Phillips. On April 28 at Maternité Grande-Duchesse Charlotte, Luxembourg, to Cynthia and Edward—a daughter (Alison Catherine), a sister for James.

London accommodation. University of London mathematician seeks responsible family for furnished house while he is on leave, 3 bedrooms, convenient for schools and public transport, Raynes Park, SW20, from July 1, 1975 to September 30, 1976 (Dr N. S. Swaminarayan, Department of Mathematics, Chelsea College, Manresa Road, London SW3 6LX, UK).

London accommodation. Two bedroom terraced house available for academic year 1975–76, 25 minutes from City centre, fully furnished, central heating, small garden (Dr J. F. Smyth, 51 Constance Road, Sutton, Surrey, UK; 01-643 8901, day; 01-642 3673, eve.)

There will be no charge for this service. Send items (not more than 60 words) to Robert Vickers at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

Reports and publications

Great Britain

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